Search Strategy

FILE 'USPATFULL' ENTERED AT 12:21:24 ON 24 JUN 2003

		E	HALLOWITZ R A/IN
L1	7	S	E4 OR E5
		E	KROWKA JOHN/IN
L2	1	S	E3
		E	MATLOCK SHAWN/IN
L3	2	S	E3 OR E4

FILE 'MEDLINE' ENTERED AT 12:28:41 ON 24 JUN 2003

		E	HALLOWITZ R A/AU
L4	4	S	E2 OR E3
		E	KROWKA J/AU
L5	38	S	E3 OR E4
		E	MATLOCK S/AU

FILE 'USPATFULL' ENTERED AT 12:32:13 ON 24 JUN 2003

L6	25015	S	(HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L7	2473	S	L6 AND (GP120)
L8	18	S	L7 AND (CELL-SURFACE GP120)
L9	16	S	L8 NOT L1
L10	299	S	L7 AND GP120/CLM
L11	172	S	L10 AND ANTIBOD?/CLM
L12	2	S	L11 AND (FLUORESCENCE RESONANCE ENERGY TRANSFER ASSAY OR FRET
L13	32	S	L11 AND (FLOW CYTOMETRY)
L14	5	S	L10 AND (FRET OR FLUORESENCE RESONANCE ENERGY TRANSFER ASSAY)
L15	9	S	L10 AND (INFECTED CELLS/CLM)
L16	76	S	L10 AND (HIV-INFECTED CELLS)
L17	67	S	L16 NOT (L1 OR L14 OR L15)
L18	0	S	L17 AND (HIV-INFECTED CELLS/CLM)
L19	2370	S	L6 AND (MAGNETIC PARTICLES OR MAGNETIC BEADS OR PARAMAGNETIC
L20	232	S	L19 AND (MAGNETIC/CLM OR PARAMAGNETIC/CLM)
L21	7	S	L20 AND GP120/CLM
L22	4	S	L21 NOT L1
L23	30	S	L20 AND (HIV/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L24	23	S	L23 NOT L21
L25	4	S	L7 AND (FRET/CLM OR FLUORESCENCE RESONANCE ENERGY TRANSFER/CL
L26	480	S	(FRET/CLM OR FLUORESCENCE RESONANCE ENERGY TRANSFER/CLM)
L27	57	S	L26 AND ANTIBOD?/CLM
L28	518	S	L6 AND (FRET OR FLUORESCENCE RESONANCE ENERGY TRANSFER)
L29	70	S	L28 AND (FRET/CLM OR FLUORESCENCE RESONANCE/CLM)
L30	49	S	L29 NOT L27
		E	ALLAWAY G P/IN
L31	16	S	E4
L32	2827	S	L6 AND FLOW CYTOMETRY
L33	104	S	L32 AND (FLOW CYTOMETRY/CLM)
L34	22	S	L33 AND (HIV/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L35	694	S	L32 AND (CELL SORTING)
L36	204	S	L35 AND (ANTI-CD4)
L37	10	S	L36 AND (ANTI-GP120)
L38	1	S	L36 AND (ANTI-CD4/CLM)
L39	1	S	L35 AND (ANTI-CD4/CLM)
L40	160	S	L32 AND (CD4 (5W) MONOCLONAL)
L41	71	S	L40 AND CD4/CLM

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L42          23 S L41 AND MONOCLONAL/CLM

     FILE 'MEDLINE' ENTERED AT 13:35:44 ON 24 JUN 2003

                E ALLAWAY G P/AU
L43          23 S E3-E5
L44           0 S L43 AND (FRET OR FLUORESCENCE RESONANCE ENERGY TRANSFER OF FL
L45        2050 S (FRET OR RESONANCE ENERGY TRANSFER)
L46          33 S L45 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L47      131782 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L48          31 S L47 AND (MAGNETIC PARTICLES OR MAGNETIC BEADS OR PARAMAGENTIC
L49          11 S L47 AND (IMMUNOMAGNETIC BEADS OR IMMUNOMAGNETIC PARTICLES)
L50           9 S L49 NOT L48
L51         499 S L47 AND (HIV-INFECTED CELLS)
L52         185 S L51 AND CD4
L53          62 S L52 AND GP120
L54           6 S L53 AND DETECT?
L55          56 S L53 NOT L54
L56         121 S L51 AND DETECT?
L57         115 S L56 NOT L53
L58           9 S L47 AND (CELL SURFACE GP120 OR GP120 SURFACE ANTIGEN OR VIRAL
L59        5445 S L47 AND GP120
L60         943 S L59 AND (QUANTIT? OR DETECT?)
L61         214 S L60 AND QUANTI?
L62        2633 S L47 AND (HIV-INFECTED CELLS OR HIV-INFECTED INDIVIDUALS)
L63         477 S L62 AND DETECT?
L64          36 S L63 AND GP120
L65        5545 S L47 AND (VIRAL LOAD OR VIRAL BURDEN)
L66         121 S L65 AND GP120
L67         120 S L66 NOT L64

     FILE 'WPIDS' ENTERED AT 14:14:15 ON 24 JUN 2003

                E HALLOWITZ R A/IN
L68           8 S E2-E3
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L1 ANSWER 6 OF 7 USPATFULL
1998:122214 Reagent system for detecting HIV-infected peripheral blood lymphocytes in whole blood.
King, Chester F., Frederick, MD, United States
Hallowitz, Robert A., Gaithersburg, MD, United States
The Avriel Group, AMCAS Division Inc., United States (part interest) a part interest
US 5817458 19981006
APPLICATION: US 1996-732782 19961015 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Fluorometric immunological assay method for detection of HIV-1 infection in which Murine anti-gp120 monoclonal antibodies coupled to paramagnetic microspheres (14) and Fluorescein conjugated anti-gp120 polyclonal antibodies IgG (16) are incubated in a few drops of whole blood diluted in 0.5 cc phosphate buffered saline (10). After incubation for 5 minutes, the HIV-infected peripheral blood lymphocytes (18) will be coated with both the Murine anti-gp120 monoclonal antibodies coupled to paramagnetic microspheres (14) and Fluorescein conjugated anti-gp120 polyclonal antibodies IgG (16) at exposed gp120 antigens (20) binding sites. At the time of measurement said HIV- infected peripheral blood lymphocytes (18) will be pulled against the wall of the measurement vessel by means of a magnetic gradient (26). The cells adhering to the vessel wall are illuminated at 488 nm monochromatic light by a focused light source (28) and the resultant emitted fluorescence is imaged, measured and recorded.

CLM What is claimed is:
1. A method of detecting an HIV-infected cell in an aqueous sample comprising the steps of, a) combining a first anti-gp120 antibody attached to a magnetic particle; a second anti-gp120 antibody attached to a detectable label; and an aqueous sample containing HIV-infected peripheral blood lymphocytes displaying gp120 on the cell surface, to form a mixture; b) incubating said mixture under conditions effective for binding of said antibodies to said gp120 to form a complex, said complex comprising said first and second antibody bound to a HIV-infected cell on said magnetic particle; and c) moving said magnetic particle to a predetermined point on a reaction vessel holding said mixture, wherein said moving is accomplished by a magnetic field acting on said magnetic particle; d) detecting the label of said second antibody bound to gp120 on said HIV-infected cell, with the proviso that no step of washing of said mixture and no step of removing unbound first antibody and unbound second antibody from said mixture is performed in steps a), b), c), and d).

2. A method of claim 1, wherein said first and second antibody recognize different regions of gp120.

3. A method of claim 1, wherein said aqueous sample is whole blood.

4. A method of claim 1, wherein said predetermined point is illuminated with a light effective to detect said label.

5. A method of claim 1, wherein said detectable label is FITC.

6. A method of claim 1, wherein said first antibody is a monoclonal antibody.

7. A method of claim 1, wherein said second antibody is a polyclonal

antibody.

8. A method of claim 1, where said first antibody is a monoclonal antibody and said second antibody is a polyclonal antibody coupled to a detectable label which is FITC.

9. A method of detecting an HIV-infected cell in an aqueous sample comprising the steps of, a) combining a first anti-gp120 antibody attached to a magnetic particle; a second anti-gp120 antibody attached to a detectable label; and an aqueous sample containing HIV-infected cells displaying gp120 on the cell surface, to form a mixture; b) incubating said mixture under conditions effective for binding of said antibodies to said gp120 to form a complex, said complex comprising said first and second antibody bound to a HIV-infected cell on said magnetic particle; and c) moving said magnetic particle to a predetermined point on a reaction vessel holding said mixture, wherein said moving is accomplished by a magnetic field acting on said magnetic particle; d) detecting the label of said second antibody bound to gp120 on said HIV-infected cell, with the proviso that no step of washing of said mixture and no step of removing unbound first antibody and second antibody from said mixture is performed in a), b), c), and d).

10. A method of claim 1, wherein said HIV-infected cell is a peripheral blood lymphocyte.

11. A method of claim 9, wherein said first and second antibody recognize different regions of gp120.

12. A method of claim 9, wherein said aqueous sample is whole blood.

13. A method of claim 9, wherein said predetermined point is illuminated with a light effective to detect said label.

14. A method of claim 9, wherein said detectable label is FITC.

15. A method of claim 9, wherein said first antibody is a monoclonal antibody.

16. A method of claim 9, wherein said second antibody is a polygonal antibody.

17. A method of claim 9, where said first antibody is a monoclonal antibody and said second antibody is a polyclonal antibody coupled to a detectable label which is FITC.

L1 ANSWER 4 OF 7 USPATFULL
2001:114495 REAGENT SYSTEM AND KIT FOR DETECTING HIV INFECTED CELLS.
KING, CHESTER F., FREDERICK, MD, United States
HALLOWITZ, ROBERT A., GAITHERSBURG, MD, United States
US 2001008760 A1 20010719
APPLICATION: US 1998-139663 A1 19980825 (9)
WO 1997-US18649 19971015 None PCT 102(e) date
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

WOULD NOT PATENT?

AB This invention relates to blood collection and diagnostics. More particularly, the invention relates to blood collection and diagnostics utilizing techniques such as magnetic separation and photodetection. The present invention also relates to methods and an apparatus for detecting the presence of antigens displayed on the surface of cells. More preferably, the present invention relates to the detection of cells

infected by human immunodeficiency virus (HIV) and related viruses. In accordance with the present invention, HIV-infected cells can be detected and separated from uninfected cells. In a preferred embodiment, separation is achieved by a magnetic field. By coating the infected cells with magnetic particles, transfer of the cells to a precise location is facilitated. A novel aspect of the present invention is a cartridge antigen test which allows for the collection and mixing of blood with reagents in one package, which can be viewed on a fluorescent microscope.

CLM What is claimed is:

1. A method of separating cells expressing a viral antigen, comprising: a) contacting a target cell with a virus capable of infecting the cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the mixture, a first binding partner specific for an antigen coded for by the virus which is expressed on the surface of the cell upon viral infection, under conditions effective for the first binding partner to bind to the viral antigen on the cell surface; c) adding to the mixture resulting from b), a second binding partner specific for the first binding partner and attached to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the first binding partner is bound to the antigen expressed by the cell, to form a complex; and d) separating target cells containing the complex, whereby said separation is achieved by a magnetic field.

2. A method of claim 1, further comprising adding to the target cell a sample antibody specific for the viral antigen.

3. A method of claim 2, further comprising measuring the number of target cells separated in d) in the presence and absence of the sample antibody

4. A method of claim 1, further comprising adding to the target cell a sample comprising an antibody specific for the viral antigen, whereby the amount of the second antibody is effective for interfering with the binding of the first binding partner to the viral antigen.

5. A method of claim 1, further comprising adding to the target cell a sample suspected of containing an antibody specific for the viral antigen.

6. A method of claim 5, further comprising measuring the number of target cells separated in d) in the presence and absence of the sample.

7. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen.

8. A method of claim 6, wherein the second binding partner is an antibody specific for the first binding partner.

9. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen, which antibody is labeled with a detectable label.

10. A method of claim 9, wherein the second binding partner is an antibody specific for the detectable label.

11. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen, which antibody is labeled with a detectable label.

12. A method of claim 6, wherein the virus is HIV.

13. A method of claim 6, wherein the first binding partner is an antibody specific for the viral antigen gp120, which antibody is labeled with a detectable label; and the second binding partner is an antibody specific for the detectable label.

14. A method of claim 6, wherein the target cell is a T-cell line.

15. A method of claim 6, wherein the sample is a body fluid or blood.

16. A method of claim 6, wherein measurement of the number of target cells separated in d) in the presence and absence of the sample is accomplished by flow cytometry.

17. A method of claim 12, wherein the first binding partner is a receptor for the viral antigen.

18. A method of claim 16, wherein the first binding partner is a receptor for the viral antigen and is labeled with a detectable label; and the second binding partner is an antibody specific for the detectable label.

19. A method of claim 6, wherein the bead diameter is about 50-120 nm.

20. A method of claim 6, wherein the cell is contacted by at least about 100-1000 beads.

21. A method of identifying an agent which interferes with viral infection of a cell, a) contacting a test cell with a virus capable of infecting the test cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the resultant mixture formed in a), a test sample containing an agent suspected with interfering with viral infection of the test cell; c) adding to the mixture of b), a first binding partner specific for an antigen coded for by the virus which is expressed on the surface of the test cell upon viral infection, under conditions effective for the binding partner to bind to the viral antigen on the cell surface; d) adding to the resultant mixture formed in c), a second binding partner specific for the first binding partner and to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the latter is bound to the viral antigen expressed by the test cell, to form a complex; e) separating test cells containing said complex, whereby said separation is achieved by a magnetic field; and f) determining the number of cells infected with said virus in the presence and the absence of said test agent.

22. A magnetic bead having a surface coated by a cell-surface virus receptor for HIV.

23. A magnetic bead of claim 21, wherein the virus receptor is CD4.

24. A method of separating virus-infected cells from non-virus infected cells in a sample comprising, combining (a) a first antibody recognizing a viral antigen on the surface of said cell and attached to a magnetic particle; (b) a second antibody recognizing said viral antigen on the surface of said cell and attached to a detectable label; and (c) a sample containing said virus-infected cells, to form a mixture; incubating said mixture under conditions effective for binding of said antibodies to said viral antigen to form a complex, said complex

comprising said first and second antibody bound to said virus-infected cell, and moving said magnetic particle to a predetermined point on a reaction vessel holding said mixture, whereby said moving is accomplished by a magnetic field acting on said magnetic particle resulting in separating said virus-infected cells from non-virus infected cells, wherein said moving is accomplished without removing unbound antibody first and second antibody from said mixture.

25. A method of claim 24, further comprising detecting the label of said second antibody bound to said viral antigen on said virus-infected cell, wherein said first and second antibody recognize different epitopes of said viral antigen.

26. A method of separating cells infected with a virus, comprising: a) contacting a target cell with a virus capable of infecting the cell, under conditions effective for achieving infection of the cell with the virus; b) fixing and permeabilizing said cells; c) adding to the fixed and permeabilized cells, a first binding partner specific for an antigen coded for by the virus, which viral antigen is ultimately expressed on the surface of the cell upon viral infection, under conditions effective for the first binding partner to bind to said viral antigen on the inside of said fixed and permeabilized cell; d) adding to the result of c), a second binding partner specific for the first binding partner and attached to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the first binding partner is bound to the antigen expressed by the cell, to form a complex; and e) separating target cells containing the complex, whereby said separation is achieved by a magnetic field.

27. A method of identifying an agent which interferes with viral infection of a cell, comprising: a) contacting a test cell with a virus capable of infecting the test cell, under conditions effective for achieving infection of the cell with the virus, to form a mixture; b) adding to the resultant mixture formed in a), a test agent suspected with interfering with viral infection of the test cell; c) fixing and permeabilizing said cells; d) adding a first binding partner specific for an antigen coded for by the virus, which viral antigen is expressed ultimately on the surface of the test cell upon viral infection, under conditions effective for the binding partner to bind to the viral antigen when said viral antigen is expressed in the interior of said cell; e) adding to the resultant mixture formed in d), a second binding partner specific for the first binding partner and to a magnetic bead, under conditions effective for the second binding partner to bind to the first binding partner, when the latter is bound to the viral antigen expressed by the test cell, to form a complex; f) separating test cells containing said complex, whereby said separation is achieved by a magnetic field; and g) determining whether the test sample changes the number of test cells containing the complex when compared to the process performed in the absence of said agent.

28. A method claim 27, where said test agent is added to cells prior to simultaneous to contacting cell with said test agent.

29. A method of separating cells expressing a cell-surface viral antigen, comprising: a) combining an effective amount of an anti-cell-surface viral antigen antibody attached to a detectable label, an effective amount of an antibody specific-for said detectable label, and an aqueous sample containing viral-infected cells displaying said cell-surface viral antigen, to form a mixture, wherein said antibody specific-for said detectable label is attached to a magnetic particle; b) incubating said mixture under conditions effective for binding of

said anti-cell surface viral antibody to said cell-surface viral antigen, and, for binding of said antibody specific-for said detectable label to said detectable label attached to said anti-cell surface viral antibody, to form a complex, wherein said anti-viral antibody is bound to said cell-surface antigen displayed on a viral-infected cell; and c) separating said complex, comprising said cells expressing said cell-surface viral antigen and magnetic particles, by applying a magnetic field to said mixture, whereby said complex is retained by said magnetic field.

30. A method of claim 29, wherein viral-infected cells are infected with HIV.

31. A method of claim 29, wherein said cell-surface viral antigen is an envelope glycoprotein for HIV.

32. A method of claim 29, wherein the envelope glycoprotein is gp120 or gp41.

33. A method of claim 29, wherein said anti-cell surface viral antibody is a polyclonal antibody specific for HIV envelope glycoprotein and said viral-infected cells are infected with HIV.

34. A method of claim 29, wherein said detectable label is FITC, TRITC, or R-phycoerthryin.

35. A method of claim 29, further comprising counting said magnetically-separated cells by flow cytometry.

36. A method of claim 29, wherein said magnetic particles are about 10-150 nm in diameter. A method of separating cells expressing a cell-surface viral antigen, comprising: a) combining an effective amount of an anti-cell-surface viral antigen antibody attached to a magnetic particle and an aqueous sample containing viral-infected cells displaying said cell-surface viral antigen, to form a mixture; b) incubating said mixture under conditions effective for binding of said anti-cell surface viral antibody to said cell-surface viral antigen displayed on said viral-infected cells, to form a complex; and c) separating said complex comprising said cells expressing said cell-surface viral antigen and magnetic particles by applying a magnetic field to said mixture, whereby said complex is retained by said magnetic field.

L1 ANSWER 3 OF 7 USPATFULL
2001:199904 METHODS AND COMPOSITIONS FOR DETERMINING LATENT VIRAL LOAD.
HALLOWITZ, ROBERT, GAITHERSBURG, MD, United States
SALAS, VIRGINIA, ALBUQUERQUE, NM, United States
US 2001039007 A1 20011108
APPLICATION: US 1999-296534 A1 19990422 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

would not present

AB The present invention relates to a new HIV status of a patient called "latent viral load." To measure the "latent viral load," in accordance with a preferred embodiment of the present invention, a population of sample cells is obtained from a desired source, such as an infected patient. The sample cell population is depleted of overtly infected cells and cells harboring active virus, to produce a subset of "resting cells" comprising uninfected and latently-infected cells. This subset is treated with an agent and/or condition that activates the latent virus in the host cell genome and results in a productive infection. The

thus-produced infection reflects the "latent viral load" of the host because it reveals the presence of quiescent virus in cells. The latent viral load is useful in assessing a patient's disease status and the efficacy of highly active antiretroviral therapy and other treatment protocols.

CLM What is claimed is:

1. A method of determining the latent viral load in a host infected with HIV comprising, treating resting lymphoid mononuclear cells obtained from the host with an effective amount of an agent capable of activating an HIV virus integrated into the genome of the cells; and detecting the expression of cell-surface gp120 after the cells have been treated with the agent, wherein the presence or amount of cells expressing cell-surface gp120 is a measure of latent viral load.

2. A method of claims 1, further comprising obtaining the resting lymphoid mononuclear cells by the steps of: a) obtaining a sample cell population; b) depleting the sample cell population of cells expressing cell-surface gp120; and c) depleting sample cell population of cells expressing HLA-DR.

3. A method of claim 2, wherein the sample cells are depleted of gp120 expressing cells by the steps of: a) contacting sample cells with gp120-specific antibodies, each conjugated to a capture moiety, under conditions effective for the antibodies to attach to gp120 on the surface of cells, thereby forming labeled-cells; b) contacting the labeled-cells with capture moiety-specific antibody under conditions effective for the capture moiety-specific antibody to attach to the labeled-cells, thereby forming a complex-labeled cells; and c) removing the complex-labeled cells, thereby depleting sample cells of gp120+ cells.

4. A method of claim 3, wherein the capture moiety-specific antibody is conjugated to magnetic particles.

5. A method of claim 3, wherein the capture moiety is FITC and the capture moiety-specific antibody is FITC-specific antibody conjugated to a magnetic bead.

6. A method of claims 4, wherein the magnetic particles are 10-100 nm in diameter.

7. A method of claims 5, wherein the magnetic particles are 10-100 nm in diameter.

8. A method of claims 3, wherein the removing is accomplished by a magnetic field acting on the magnetic particles.

9. A method of claim 2, further comprising: separating CD4+ cells from the sample.

10. A method of claim 2, further comprising: separating CD8+ cells from the sample.

L1 ANSWER 1 OF 7 USPATFULL
2002:185564 Methods for characterizing the viral infectivity status of a host.
Hallowitz, Robert A., Newmarket, MD, UNITED STATES
Krowka, John, Frederick, MD, UNITED STATES
Matlock, Shawn, Frederick, MD, UNITED STATES
Bio-Tech Imaging, Inc., Frederick, MOLDOVA, REPUBLIC OF (U.S. corporation)
US 2002098476 A1 20020725

APPLICATION: US 2001-893604 A1 20010629 (9)
PRIORITY: US 2000-215075P 20000630 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods in accordance with the present invention involve novel measurements of the disease status of hosts infected with the human immunodeficiency virus. In particular, the present invention relates to a measurements of the numbers in a sample volume of (a) productively HIV-infected cells and (b) cells capable of being infected by HIV, e.g., cells expressing CD4, CCR5, and/or CXCR4. These two values can be represented as a single ratio, e.g., number of productively infected cells/number of cells capable of being infected by HIV, and can be utilized as an indicator of disease status, such as disease progression, viral replication, etc.

CLM What is claimed is:

1. A method of assessing the infectivity status of a host infected with HIV, comprising: measuring the number of cells in a sample which are expressing cell-surface gp120 and the number of lymphocytes in said sample which are CD4 positive, whereby the infectivity status of the host is assessed.

2. A method of claim 1, wherein the infectivity status is represented by the number of cells expressing cell-surface gp120 per unit volume divided by the number of cells which are CD4 positive per unit volume.

3. A method of claim 1, wherein the measuring is accomplished by flow cytometry.

4. A method of claim 1, wherein the measuring is accomplished by a fluorescence resonance energy transfer assay.

5. A method of claim 1, wherein the cells are peripheral blood mononuclear cells.

6. A method of claim 1, further comprising: combining an effective amount of an anti-gp120 antibody attached to a first detectable label and an effective amount of an anti-CD4 antibody attached to a second detectable label under conditions effective for said antibodies to bind gp120 and CD4 respectively.

7. A method of claim 6, wherein said measuring is accomplished by flow cytometry.

8. A method of claim 1, further comprising: combining an effective amount of an anti-gp120 antibody attached to a detectable label, an effective amount of an antibody specific-for said detectable label, and an aqueous sample containing viral-infected cells displaying said gp120 to form a mixture, wherein said antibody specific-for said detectable label is attached to a magnetic particle; incubating said mixture under conditions effective for binding of said anti-gp120 antibody to gp120 on said cells, and, for binding of said antibody specific-for said detectable label to said detectable label attached to said anti-gp120 antibody, to form a complex, wherein said anti-gp120 antibody is bound to said gp120 displayed on a viral-infected cell; separating said complex by applying a magnetic field to said mixture, whereby said complex is retained by said magnetic field, and determining the presence of magnetically-separated cells by detecting said detectable label, whereby said magnetically separated cells are lymphocytes expressing cell-surface gp120.

9. A method of claim 1, wherein the CD4 count of said host is less than 200/mm.sup.3 of whole blood.

10. A method of claim 1, wherein the host has been treated with HAART.

11. A method of determining the infectivity status of a host infected with HIV virus who has tested negative in a virus co-culture assay, comprising: measuring the fraction of lymphocytes expressing cell-surface gp120 and the fraction of lymphocytes which are CD4 positive, whereby the infectivity status of the host is assessed.

12. A method of claim 11, wherein the measuring is accomplished by flow cytometry.

13. A method of claim 11, wherein the measuring is accomplished by a fluorescence resonance energy transfer assay.

14. A method of claim 11, wherein the cells are peripheral blood mononuclear cells.

15. A method of claim 11, further comprising: combining an effective amount of an anti-gp120 antibody attached to a first detectable label and an effective amount of an anti-CD4 antibody attached to a second detectable label under conditions effective for said antibodies to bind gp120 and CD4 respectively.

16. A method of claim 15, wherein said measuring is accomplished by flow cytometry.

L13 ANSWER 25 OF 32 USPATFULL
1998:162657 Monoclonal antibodies which neutralize HIV-1 infection.
Chang, Tse Wen, Houston, TX, United States
Fung, Michael S. C., Houston, TX, United States
Sun, Bill N. C., Wellfort Bellaire, TX, United States
Sun, Cecily R. Y., Wellfort Bellaire, TX, United States
Chang, Nancy T., Houston, TX, United States
Tanox, Inc., Houston, TX, United States (U.S. corporation)
US 5854400 19981229
APPLICATION: US 1992-950571 19920922 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Murine monoclonal antibodies and related products such as antibody fragments, immunotoxins, human and humanized antibodies are disclosed, all of which bind to the gp120 protein on the envelope of HIV-1. These antibodies and related products neutralize HIV-1. They inhibit the infection of T cells, and also inhibit syncytium formation. Further, the antibodies are preferably group-specific and neutralize various strains and isolates of HIV-1. These antibodies have a variety of uses, including the treatment of AIDS and ARC, the prevention of HIV-1 infection, as well as a diagnostic application, in that they can be used for assaying of unknown fluid samples for HIV-1.

CLM What is claimed is:
1. A composition comprising monoclonal antibodies or antigen binding fragments thereof which bind to a peptide of the same sequence as the sequence of the amino acid residue numbers 298 to 322 of gp120 of HIV-1.

2. A composition comprising monoclonal antibodies or antigen binding fragments thereof which bind to a peptide of the same sequence as SEQ ID NO: 1.

3. A monoclonal antibody or an antigen binding fragment thereof which binds to a peptide of the same sequence as the sequence of amino acid residue numbers 308 to 322 of gp120 of HIV -1.

4. A monoclonal antibody or an antigen binding fragment thereof which binds to a peptide of the same sequence as SEQ ID NO: 3.

5. A monoclonal antibody or an antigen binding fragment thereof or antigen binding fragments thereof which binds to a peptide of the same sequence as the sequence of amino acid residue numbers 298 to 312 of gp120 of HIV-1.

6. A monoclonal antibody or an antigen binding fragment thereof which binds to a peptide of the sequence of SEQ ID NO: 2.

7. A monoclonal antibody or an antigen binding fragment thereof which binds to a peptide of the same sequence as the sequence of amino acid residue numbers 169 to 183 of gp120 of HIV -1.

8. A monoclonal antibody or an antigen binding fragment thereof which binds to a peptide of the sequence of SEQ ID NO: 4.

9. The monoclonal antibodies BAT 123, BAT 267 and BAT 085 or antigen binding fragments thereof.

10. The cell lines producing the monoclonal antibodies BAT 123, BAT 267 and BAT 085 or antigen binding fragments thereof.

L13 ANSWER 28 OF 32 USPATFULL
1998:9594 HIV monoclonal antibody specific for the HTLV-III.sub.mn gp120 envelope glycoprotein.
Eda, Yasuyuki, Kumamoto-ken, Japan
Osatomi, Kiyoshi, Nagasaki, Japan
Shiosaki, Kouichi, Kumamoto-ken, Japan
Tokiyoshi, Sachio, Kumamoto, Japan
Matsushita, Shuzo, Kumamoto, Japan
Hattori, Toshio, Kyoto, Japan
Takatsuki, Kiyoshi, Kumamoto, Japan
Juridical Foundation The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan (non-U.S. corporation)
US 5712373 19980127
APPLICATION: US 1994-253030 19940602 (8)
PRIORITY: JP 1990-175075 19900702
JP 1990-188300 19900716
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A murine monoclonal antibody which specifically binds to a glycoprotein antigen having a molecular weight of about 12.times.10.sup.4 daltons (gp120) present in the envelope of human T-lymphotropic virus III.sub.MN (HTLV-III.sub.MN) and capable of neutralizing the HTLV-III.sub.MN as determined by in vitro inhibition of syncytium formation but does not bind to other HTLV-III strains, or antigen-binding fragments, which is useful for prophylaxis, treatment and diagnosis of AIDS.

CLM What is claimed is:

1. A murine monoclonal antibody which is produced by the hybridoma FERM BP-3402 and which is capable of neutralizing human T-lymphotropic virus III.sub.MN (HTLV-III.sub.MN) and has the following characteristics: (a) immunoglobulin class: IgG, K, (b) specifically binds to an epitope which is present in the region represented by the amino acid sequence 303 to 325 (Tyr Ash Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys Asn Ile Ile Gly) of a glycoprotein antigen having a molecular weight of about 12.times.10.sup.4 daltons of gp120 of HTLV-III.sub.MN, SEQ ID NO:1, (c) specifically binds to the surface of HTLV-III.sub.MN viral particles and thereby inhibits the infection of CD4-positive cells by HTLV-III.sub.MN, (d) specifically binds to the surface of cells infected with HTLV-III.sub.MN and thereby inhibits the syncytium formation induced by interaction between the infected cells and uninfected cells as determined by in vitro inhibition of syncytium formation, and (e) does not bind to any HTLV-III strain other than HTLV-III.sub.MN, or an antigen-binding fragment thereof.

L13 ANSWER 29 OF 32 USPATFULL
97:115093 Monoclonal antibodies specific for HIV and the hybridomas for production thereof.
Masuho, Yasuhiko, Tokyo, Japan
Sugano, Toru, Tokyo, Japan
Matsumoto, Yoh-ichi, Kichijohjikita-machi, Japan
Kawamura, Takashi, Tokyo, Japan
Hersh, Evan, Tucson, AZ, United States
Petersen, Eskild, Tucson, AZ, United States
Lake, Douglas, Tucson, AZ, United States
The University of Arizona, Department of Internal Medicine, Section of Hematology and Oncology, Tucson, AZ, United States (U.S. corporation)Teijin Limited, Osaka, Japan (non-U.S. corporation)
US 5695927 19971209
APPLICATION: US 1994-270214 19940701 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Human monoclonal antibodies which belong to the IgG1 subclass and are specific for HIV are described. The monoclonal antibodies have potential for use in the diagnosis, prevention and therapy of HIV infection.

CLM What is claimed is:

1. A method for blocking and preventing the binding of viral or recombinant gp120 glycoprotein to lymphocyte CD4 or soluble CD4 in vitro, comprising the step of: contacting said gp120 glycoprotein with human monoclonal IgG1 antibodies produced by hybridoma ATCC Accession No. HB10074 which immunologically bind to said gp120 and prevent the binding of said gp120 to said CD4.

2. A method for blocking and preventing the binding of recombinant gp120 glycoprotein to soluble CD4 in vitro, comprising the step of: contacting said gp120 glycoprotein with human monoclonal IgG1 antibodies produced by hybridoma ATCC Accession No. HB10074 which immunologically bind to said gp120 preventing the binding of said gp120 to said CD4 to form antibody /gp120 immune complexes.

3. A method for neutralizing a human immunodeficiency virus (HIV) in vitro, comprising the step of:

contacting an HIV with human monoclonal IgG1 antibodies produced by hybridoma ATCC Accession No. HB10074 which immunologically bind to gp120 glycoprotein on the surface of said HIV and prevent binding of gp120 glycoprotein to lymphocyte CD4.

4. A method for screening a serum sample for antibodies capable of blocking and inhibiting the binding of viral or recombinant gp120 glycoprotein of human immunodeficiency virus type 1 (HIV-1) to lymphocyte CD4 or soluble CD4 in vitro, comprising the steps of: a) contacting a first portion of said gp120 glycoprotein with a serum sample in a manner and for a time sufficient to allow formation of gp120/antibody immune complexes; b) attaching soluble CD4 to a solid support; c) blocking said attached CD4 with a nonspecific binding protein to form bound blocked CD4; d) contacting said bound blocked CD4 with the gp120/antibody immune complexes of step a) in a manner and for a time sufficient to allow formation of bound CD4/gp120 /antibody immune complexes; e) contacting said bound CD4/ gp120/antibody immune complexes with labeled anti-gp120 antibodies; f) detecting said label wherein detection of the label determines the presence of antibodies blocking gp120/CD4 binding; g) contacting a second portion of said gp120 glycoprotein with human monoclonal IgG1 antibodies produced by hybridoma ATCC Accession No. HB10074 which immunologically bind to said gp120 and prevent binding of said gp120 to said CD4 to form HB10074 antibody/ gp120 immune complexes; h) attaching soluble CD4 to a second solid support; i) blocking said CD4 attached to said second solid support with nonspecific binding protein to form blocked CD4 bound to said second solid support; j) contacting said blocked CD4 bound to said second solid support with said HB10074 antibody/gp120 immune complexes in a manner and for a time sufficient to allow formation of bound CD4/gp120/HB10074 antibody immune complexes; k) contacting said bound CD4/gp120/antibody immune complexes with labeled anti-gp120 antibodies; l) detecting said label wherein detection of the label determines the presence of HB10074 antibodies blocking gp120/CD4 binding; and m) comparing the amount of label detected in step f) with the amount of label detected in step l).

5. The method of claim 4, wherein said label is a chromogenic or fluorogenic enzyme, which is detected by contacting said enzyme with a chromogenic substrate.

6. The method of claim 5, wherein said enzyme is horseradish peroxidase and said substrate is tetramethylbenzidine.

L15 ANSWER 8 OF 9 USPATFULL
1998:9594 HIV monoclonal antibody specific for the HTLV-III.sub.mn gp120 envelope glycoprotein.
Eda, Yasuyuki, Kumamoto-ken, Japan
Osatomi, Kiyoshi, Nagasaki, Japan
Shiosaki, Kouichi, Kumamoto-ken, Japan
Tokiyoshi, Sachio, Kumamoto, Japan
Matsushita, Shuzo, Kumamoto, Japan
Hattori, Toshio, Kyoto, Japan
Takatsuki, Kiyoshi, Kumamoto, Japan
Juridical Foundation The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan (non-U.S. corporation)
US 5712373 19980127

APPLICATION: US 1994-253030 19940602 (8)
PRIORITY: JP 1990-175075 19900702
JP 1990-188300 19900716
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A murine monoclonal antibody which specifically binds to a glycoprotein antigen having a molecular weight of about 12.times.10.sup.4 daltons (gp120) present in the envelope of human T-lymphotropic virus III.sub.MN (HTLV-III.sub.MN) and capable of neutralizing the HTLV-III.sub.MN as determined by in vitro inhibition of syncytium formation but does not bind to other HTLV-III strains, or antigen-binding fragments, which is useful for prophylaxis, treatment and diagnosis of AIDS.

CLM What is claimed is:
1. A murine monoclonal antibody which is produced by the hybridoma FERM BP-3402 and which is capable of neutralizing human T-lymphotropic virus III.sub.MN (HTLV-III.sub.MN) and has the following characteristics: (a) immunoglobulin class: IgG, K, (b) specifically binds to an epitope which is present in the region represented by the amino acid sequence 303 to 325 (Tyr Ash Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys Asn Ile Ile Gly) of a glycoprotein antigen having a molecular weight of about 12.times.10.sup.4 daltons of **gp120** of HTLV-III.sub.MN, SEQ ID NO:1, (c) specifically binds to the surface of HTLV-III.sub.MN viral particles and thereby inhibits the infection of CD4-positive cells by HTLV-III.sub.MN, (d) **specifically binds to the surface of cells infected with HTLV-III.sub.MN** and thereby inhibits the syncytium formation induced by interaction between the infected cells and uninfected cells as determined by in vitro inhibition of syncytium formation, and (e) does not bind to any HTLV-III strain other than HTLV-III.sub.MN, or an antigen-binding fragment thereof.

L17 ANSWER 51 OF 67 USPATFULL
1998:86038 Monoclonal antibodies specific for HIV and the hybridomas for production thereof.
Masuho, Yasuhiko, Tokyo, Japan
Sugano, Toru, Tokyo, Japan
Matsumoto, Yoh-ichi, Tokyo, Japan
Kawamura, Takashi, Tokyo, Japan
Hersh, Evan, Tucson, AZ, United States
Petersen, Eskild, Tucson, AZ, United States
Lake, Douglas, Tucson, AZ, United States
The University of Arizona, Department of Internal Medicine, Section of Hematology and Oncology, Tucson, AZ, United States (U.S. corporation)Teijin Limited, Osaka, Japan (non-U.S. corporation)
US 5783670 19980721
APPLICATION: US 1995-487529 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Human monoclonal antibodies which belong to the IgG1 subclass and are specific for HIV are described. The monoclonal antibodies have potential for use in the diagnosis, prevention and therapy of HIV infection.

CLM What is claimed is:
1. A method for neutralizing a human immunodeficiency virus type 1 (HIV-1), comprising: contacting an HIV-1 with human monoclonal IgG1 antibodies produced by

hybridoma ATCC Accession No. HB10074 which immunologically bind to gp120 glycoprotein on the surface of said HIV-1 and prevent binding of gp120 glycoprotein to lymphocyte CD4.

2. The method of claim 1, wherein said antibodies are produced by a subcloned cell line obtained from hybridoma ATCC Accession No. HB10074 by limiting dilution.

L17 ANSWER 59 OF 67 USPATFULL
97:66028 Human neutralizing monoclonal antibodies to human immunodeficiency virus.
Burton, Dennis R., La Jolla, CA, United States
Barbas, Carlos F., San Diego, CA, United States
Lerner, Richard A., La Jolla, CA, United States
The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)
US 5652138 19970729
APPLICATION: US 1994-276852 19940718 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention describes human monoclonal antibodies which immunoreact with and neutralize human immunodeficiency virus (HIV). Also disclosed are immunotherapeutic and diagnostic methods of using the monoclonal antibodies, as well as cell line for producing the monoclonal antibodies.

CLM What is claimed is:
1. A human monoclonal antibody that immunoreacts with human immunodeficiency virus (HIV) glycoprotein gp120, neutralizes HIV, and is produced by the cell line designated MT12 having A.T.C.C. Accession Number 69079.

2. A human monoclonal antibody comprising the variable region amino acid sequence of the human monoclonal antibody produced by the cell line designated MT12 having A.T.C.C. Accession Number 69079.

3. A cell line designated MT12 having A.T.C.C. Accession Number 69079.

L24 ANSWER 20 OF 23 USPATFULL
97:22466 Diagnosis and treatment of HIV viral infection using magnetic metal transferrin particles.
Gordon, deceased, David, late of Skokie, IL, United States
Gordon, legal representative, by Eunice, 4936 W. Estes, Skokie, IL, United States 60077
Gordon, Robert T., 4936 W. Estes, Skokie, IL, United States 60077
US 5612019 19970318
APPLICATION: US 1994-195070 19940214 (8)
DOCUMENT TYPE: Utility; Granted.

AB This invention provides methods of treatment and/or diagnosis and/or siting of viruses including the AIDS virus and others as well as the cells which they infect. The method comprises introducing near, into or onto the virus or the cell which the virus infects, or both, minute particles. These particles possess ferromagnetic, paramagnetic or diamagnetic properties. After being localized near, in or on the virus or the viral-infected cell, the particles are inductively heated by application of an alternating electromagnetic field. The inductive heating is continued for a period of time sufficient to bring about a temperature rise to a minimum necessary to kill the virus or cell or to desirably alter the behavior of the virus or infected cell. Prior to,

during or after treatment, these particles can be used diagnostically to locate and/or map the virus in the living tissue.

CLM What is claimed is:

1. A method of diagnosing HIV viral disease or an **HIV viral infection in a cell** of or from a living host comprising: introducing into the host of said virus or cells, particles localizable near, in or on the virus or vital-infected cells, such particles being capable of responding to a magnetic field and being of a size less than 1 micron, whereby the particles are selectively located near, into or on the virus and/or viral-infected cells, wherein said particles are magnetic metal transferrin particles, and measuring a magnetic property of the particles.

2. A method of claim 1 wherein said measuring provides a map which identifies the location and/or the concentration of the particles.

3. A method according to claim 2 wherein a SQUID magnetometer is used for the magnetic mapping.

4. A method according to claim 2 wherein nuclear magnetic resonance is used to map the particles.

5. A method according to claim 2 wherein EPR or ESR is used to map the particles.

6. A method according to claim 1 wherein said virus is an AIDS-causing virus.

7. A method according to claim 1 wherein said particles comprise a metal which is cobalt, zinc, chromium, nickel, platinum, manganese, a rare earth metal, dysprosium, erbium, gallium, holmium, samarium, terbium, thulium, ytterbium, yttrium or iron.

8. A method of claim 1 wherein said magnetic particle is ferromagnetic, paramagnetic or diamagnetic.

9. A method of claim 1 wherein the magnetic particle transferrin is bound to dextran.

10. A method of claim 1 wherein the particle is Fe.sub.3 O.sub.4 -dextran-transferrin.

L31 ANSWER 1 OF 16 USPATFULL
2003:64649 METHODS FOR USING RESONANCE ENERGY TRANSFER- BASED ASSAY OF HIV-1 ENVELOPE GYLCOPROTEIN-MEDIATED MEMBRANE FUSION, AND KITS FOR PRACTICING SAME.
ALLAWAY, GRAHAM P., MORETON MERSEYSIDE, UNITED KINGDOM
LITWIN, VIRGINIA M., FAYETTEVILLE, NY, UNITED STATES
MADDON, PAUL J., ELMSFORD, NY, UNITED STATES
US 2003044770 A1 20030306
APPLICATION: US 1999-412284 A1 19991005 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides: agents determined to be capable of specifically inhibiting the fusion of a macrophage-tropic primary isolate of HIV-1 to a CD4.sup.+ cell, but not a T cell-tropic isolate of HIV-1 to a CD4.sup.+ cell; and agents determined to be capable of specifically inhibiting the fusion of a T cell-tropic isolate of HIV-1 to a CD4.sup.+ cell, but not a macrophage-tropic primary isolate of HIV-1 to a

CD4.sup.+ cell. This invention also provides: agents capable of specifically inhibiting the fusion of a macrophage tropic primary isolate of HIV-1 with a CD+ cell susceptible to infection by a macrophage-tropic primary isolate of HIV-1; and agents capable of specifically inhibiting the fusion of a T cell-tropic isolate of HIV-1 with a CD4.sup.+ cell susceptible to infection by a T cell-tropic isolate of HIV-1. The agents include but are not limited to antibodies. This invention further provides: methods of inhibiting fusion of a macrophage-tropic primary isolate of HIV-1 with a CD+ cell susceptible to infection by a macrophage-tropic primary isolate of HIV-1 which comprises contacting the CD4.sup.+ cell with an amount of an agent capable of specifically inhibiting such fusion so as to thereby inhibit such fusion; and methods of inhibiting fusion of a T cell-tropic isolate of HIV-1 with a CD4.sup.+ cell susceptible to infection by a T cell-tropic isolate of HIV-1 which comprises contacting the CD4.sup.+ cell with an amount of an agent capable of specifically inhibiting such fusion so as to thereby inhibit such fusion.

CLM What is claimed is:

1. A method for determining whether an agent: is capable of inhibiting the fusion of- a macrophage-tropic primary isolate of HIV-1 to a CD4.sup.+ cell which comprises: (a) contacting (i) an appropriate CD4.sup.+ cell, which is labeled with a first dye, with (ii) a cell expressing the HIV-1 envelope glycoprotein of the macrophage-tropic primary isolate of HIV-1 on its surface, which is labeled with a second dye, in the presence of an excess of the agent under conditions permitting the fusion of the CD4.sup.+ cell to the cell expressing the HIV-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow resonance energy transfer between the dyes; (b) exposing the product of step (a) to conditions which would result in resonance energy transfer if fusion has occurred; and (c) determining whether there is a reduction of resonance energy transfer, when compared with the resonance energy transfer in the absence of the agent, a decrease in transfer indicating that the agent is capable of inhibiting fusion of HIV-1 to CD4.sup.+ cells.

2. The method of claim 1, wherein the CD4.sup.+ cell is a PM1 cell, a primary human T lymphocyte, or a primary human macrophage.

3. The method of claim 1, wherein the HIV-1 envelope glycoprotein.sup.+ cell is an HIV-1.sub.JR-FL gp120/gp41 HeLa cell.

4. The method of claim 1 wherein the agent is not previously known.

5. An agent determined to be capable of inhibiting the fusion of a macrophage-tropic primary isolate of HIV-1 to a CD4.sup.+ cell using the method of claim 1.

6. A therapeutic agent capable of inhibiting the fusion of an HIV-1 envelope glycoprotein.sup.+ cell with an appropriate CD4.sup.+ cell using the method of claim 1.

L34 ANSWER 12 OF 22 USPATFULL
2001:93281 Methods for measuring viral infectivity.
Hutchins, Beth M., San Diego, CA, United States
Nunnally, Mary H., Highlands Ranch, CO, United States
Sugarman, Barry J., San Diego, CA, United States
Canji, Inc., San Diego, CA, United States (U.S. corporation)
US 6248514 B1 20010619
APPLICATION: US 1998-136327 19980818 (9)
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The instant invention addresses the need for a more accurate method of quantitating infectious viral particles in a population. The methods of the instant invention are based on the unexpected and surprising result that flow cytometry analysis of cells infected using specified ranges of viral particle concentration and/or adsorption time yields a more accurate measurement of infectious virus titer than traditional titration methods.

CLM What is claimed is:

1. A method of determining the ratio of total virus particles to infectious virus particles in a viral preparation, the method comprising: a) determining the number of infectious virus particles in the viral preparation by a method that comprises: i) infecting cells in a cell population with virus particles by contacting the cells with a portion of the viral preparation, wherein the virus particles contacting the cells are present at a concentration of about 2.times.10.sup.7 particles/ml or less; ii) reacting a polypeptide expressed by the virus particles in infected cells with an antibody labeled with a fluorescent tag, the antibody having specificity for a polypeptide expressed by the virus; and iii) measuring immunofluorescence in the product of step (ii) by flow cytometry to determine the number of infected cells, thereby determining the number of infectious virus particles in the portion of the viral preparation; b) determining the total number of virus particles in the portion of the viral preparation; and c) calculating the ratio of total virus particles to infectious virus particles in the viral preparation.

2. The method of claim 1, wherein the concentration of virus particles is about 10.sup.6 particles/ml or less.

3. The method of claim 2, wherein the concentration of virus particles is about 10.sup.5 particles/ml.

4. The method of claim 1, wherein the concentration of virus particles is about 10.sup.5 particles/ml or greater.

5. The method of claim 1, wherein the cells are contacted with the preparation of virus particles for at least about five minutes.

6. The method of claim 5, wherein the cells are contacted with the preparation of virus particles for at least about thirty minutes.

7. The method of claim 5, wherein the cells are contacted with the preparation of virus particles for at least about one hour.

8. The method of claim 1, wherein the cells are contacted with the preparation of virus particles for about four hours or less.

9. The method of claim 8, wherein the cells are contacted with the preparation of virus particles for about three hours or less.

10. The method of claim 8, wherein the cells are contacted with the preparation of virus particles for about two hours or less.

11. The method of claim 1, wherein the cells are fixed after infecting the cells with the virus particles.

12. The method of claim 1, wherein the cells are present as a confluent monolayer on a surface of a container in which the infection step is

performed.

13. The method of claim 12, wherein the container is a well of a microtiter plate.

14. The method of claim 12, wherein the cells are seeded into the container at a density of about 10.sup.5 cells/cm.sup.2.

15. The method of claim 1, wherein the virus is adenovirus.

16. The method of claim 15, wherein the viral polypeptide is hexon.

17. The method of claim 1, wherein the cells are cultured after infection to allow expression of the viral polypeptide.

18. The method of claim 1, wherein the virus is a recombinant virus.

19. The method of claim 1, wherein the viral polypeptide is encoded by an exogenous gene.

20. The method of claim 19, wherein the exogenous gene is a reporter gene.

21. The method of claim 19, wherein the exogenous gene is p53.

22. The method of claim 19, wherein the exogenous gene is retinoblastoma.

23. The method of claim 1, wherein the antibody is a mixture of antibodies.

24. The method of claim 1, wherein the antibody is polyclonal.

25. The method of claim 1, wherein the antibody is monoclonal.

26. The method of claim 1, wherein the virus is replication defective.

27. The method of claim 1, wherein the virus particles are selected from the group consisting of adenovirus, adeno-associated virus, retrovirus, herpes simplex virus, parvovirus, Epstein Barr virus, rhinotracheitis virus, parainfluenza virus, parvovirus, bovine viral diarrhea virus, sindbis virus, baculovirus, pseudorabies virus, varicella-zoster virus, cytomegalovirus, HIV, hepatitis A, B, and C viruses, and vaccinia virus.

28. The method of claim 27, wherein the virus particles are adenoviral particles or retroviral particles.

L34 ANSWER 22 OF 22 USPATFULL
92:34064 CD44 as a marker for HIV infection.
Landay, Alan, 1005 N. East Ave., Oak Park, IL, United States
US 5108904 19920428
APPLICATION: US 1990-499144 19900326 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for discriminating between patients who are seropositive but asymptomatic for HIV infection and AIDS patients is disclosed wherein the fluorescence intensity from CD.sub.44.sup.+ T cells is measured. Intensity increases as AIDS progresses.

CLM What is claimed is:

1. A method for monitoring an HIV positive patient over time for conversion to AIDS comprising the steps of obtaining samples of whole blood over time from the patient, labelling cells in each sample of blood from the patient with a fluorescently labelled anti-CD44 monoclonal antibody and a fluorescently labelled anti-CD8 monoclonal antibody, measuring fluorescence intensity of CD44 on CD8+ cells in each sample by flow cytometry means and monitoring changes between samples over time in fluorescence intensity of CD44 on CD8+ cells.

2. The method of claim 1 wherein one of the fluorescent labels is phycoerythrin.

3. The method of claim 1 wherein one of the fluorescent labels is fluorescein isothiocyanate.

4. The method of claim 1 wherein the red cells in the whole blood are removed prior to measuring the fluorescence intensity.

5. The method of claim 4 wherein red cells are removed by lysis.

6. A method for monitoring an HIV positive patient over time for conversion to AIDS comprising the steps of obtaining samples of whole blood overtime from the patient, separating each sample into more than one aliquot, labelling cells in one aliquot from each sample of blood from the patient with a fluorescently labelled anti-CD8 monoclonal antibody and a fluorescently labelled anti-CD44 monoclonal antibody and in a second aliquot with a fluorescently labelled anti-CD8 monoclonal antibody and with a fluorescently labelled monoclonal second antibody selected from the group consisting of anti-CD18 and anti-CD54, measuring fluorescence intensity of CD44 and CD18 or CD54 on CD8+ cells in each aliquot respectively in each sample by flow cytometry means and monitoring changes between samples over time in fluorescence intensity of CD44 and CD18 or CD54 on CD8+ cells.

7. The method of claim 6 wherein one of the fluorescent labels is phycoerythrin.

L42 ANSWER 22 OF 23 USPATFULL
95:9617 Methods for detection and quantification of cell subsets within subpopulations of a mixed cell population.
Melnicoff, Meryle J., Cherry Hill, NJ, United States
Jensen, Bruce D., Schwenksville, PA, United States
Muirhead, Katharine A., West Chester, PA, United States
Horan, Paul K., Downingtown, PA, United States
Summers, Martin D., West Chester, PA, United States
Wong, William, Chadsford, PA, United States
Zynaxis, Inc., Malvern, PA, United States (U.S. corporation)
US 5385822 19950131
APPLICATION: US 1990-619838 19901129 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The presence or quantity of a selected subset of cells, which is part of a subpopulation of a mixed cell population, is determined by a method in which a detectable reporter substance is uniformly incorporated into substantially all cells of the subpopulation containing the subset of interest. The subset of interest is then affinity-separated by incubating a test sample of the mixed cell population containing the labeled subpopulation with a specific binding substance which

selectively binds to characteristic determinants of the cell subset of interest. Occurrence of the reporter substance in the separated fraction is then detected, and correlated to a predetermined standard to determine the presence or quantity of the subset of interest within the cell population. According to another aspect of the invention a method is provided for quantitating two or more selected subsets of cells within a subpopulation of a mixed cell population. After labeling, the entire subpopulation is affinity-separated from the mixed cell population, and occurrence of the reporter substance in the separated fraction is detected. Next, subsets of interest within the subpopulation are affinity-separated as described above, and the level of detected reporter substance in each subset is compared to the level detected in the entire subpopulation. According to further aspects of the invention test kits are provided for performing the above-described methods.

CLM What is claimed is:

1. A method for determining the presence or quantity of a selected subset of a subpopulation of cells within a mixed cell population containing said subset, said subpopulation of cells having at least one characteristic determinant and said subset of cells having at least one characteristic determinant, said method comprising: (i) selectively binding to the cells of said subpopulation a detectable reporter substance, either by a) directly binding a specific binding substance which specifically binds to said at least one characteristic determinant of said subpopulation and which is directly or indirectly conjugated to said detectable reporter substance or b) sequentially binding a first specific binding substance which specifically binds to said at least one characteristic determinant of said subpopulation, and a second specific binding substance which specifically binds to said first specific binding substance and which is directly or indirectly conjugated to said detectable reporter substance, such that a consistent amount of reporter substance is distributed in the subset of interest; (ii) contacting a test sample of said mixed cell population with a specific binding substance which specifically binds to said at least one characteristic determinant of said selected subset, resulting in the formation of a complex of cells and uncomplexed cells, said complex comprising said selected subset and the specific binding substance that binds to said subset; (iii) separating said complex from said uncomplexed cells; and (iv) following the completion of Steps (i), (ii), and (iii), detecting the occurrence of said detectable reporter substance in one of said complex or uncomplexed cells, from which the presence or quantity of said selected subset in said mixed cell population is determined.

2. A method according to claim 1, wherein said subpopulation comprises lymphocytes and said subset comprises lymphocytes characterized by selected functions or stages of differentiation.

3. A method according to claim 1, wherein said subpopulation comprises T-lymphocytes and said subset is selected from the group consisting of helper T lymphocytes and suppressor/cytotoxic T lymphocytes.

4. A method according to claim 1, wherein said subpopulation comprises leukocytes and said subset is selected from the group consisting of lymphocytes, monocytes and granulocytes.

5. A method according to claim 1, which includes the step of relating the level of detected reporter substance to a predetermined standard to determine the quantity of said subset of cells in said sample.

6. A method according to claim 1, wherein the level of detected reporter substance is related to a standard containing a predetermined quantity

of said reporter substance thereby to determine the number of said subset of cells in said sample.

7. A method according to claim 1, wherein said reporter substance is separated from said complex prior to detecting its presence or quantity.

8. A method according to claim 1, wherein said specific binding substance, which specifically binds to a determinant of said subpopulation, comprises at least one antibody.

9. A method according to claim 8, wherein said reporter substance is attached to one member of a specific binding pair, said antibody is attached to the other member of said specific binding pair, and said selective binding is achieved by the combined effect of immunological binding and binding between the members of said specific binding pair.

10. A method according to claim 9, wherein said specific binding pair comprises avidin and biotin.

11. A method according to claim 10, wherein said biotin is attached to said antibody by a cleavable linkage.

12. A method according to claim 8, wherein said at least one antibody comprises a monoclonal antibody.

13. A method according to claim 12, wherein said monoclonal antibody specifically binds to CD45 antigen.

14. A method according to claim 13 wherein the amount of said monoclonal antibody bound per cell to lymphocytes is at least 5 times greater than the amount bound per cell to monocytes.

15. A method according to claim 12, wherein said at least one antibody comprises a monoclonal antibody that specifically binds T lymphocytes.

16. A method according to claim 15, wherein said at least one antibody comprises a monoclonal antibody which specifically binds to an antigen selected from the group consisting of CD2, CD3, CD5, CD7 or any combination of said monoclonal antibodies.

17. A method according to claim 1, wherein said reporter substance comprises an enzyme.

18. A method according to claim 1, wherein said enzyme is beta-galactosidase.

19. A method according to claim 1, wherein the step of selectively binding said detectable reporter substance to said subpopulation of cells comprises sequentially binding to the surface of the cells, a first antibody which specifically binds to at least one determinant which is common to the cells of said subpopulation, and a second antibody which specifically binds to a determinant of said first antibody, and which is conjugated to said reporter substance.

20. A method according to claim 19, wherein said first antibody comprises a selected isotype and said second antibody specifically binds to a determinant of said isotype.

21. A method according to claim 19, wherein said first antibody comprises immunoglobulin obtained from a selected species and said

second antibody specifically binds to a determinant of immunoglobulin from said selected species.

22. A method according to claim 1, wherein said specific binding substance that binds to said subsets is affixed to a solid phase.

23. A method according to claim 22, wherein said solid phase comprises magnetic or paramagnetic material and said complex is separated by magnetic separation.

24. A method according to claim 22, wherein said test sample is contacted in a container with said specific binding substance which specifically binds to said selected subset and said specific binding substance which specifically binds to said selected subset comprises antibody affixed to a surface of said container in contact with said test sample.

25. A method according to claim 1, wherein said specific binding substance to which said selected subset binds comprises an antibody.

26. A method according to claim 25, wherein said antibody comprises a monoclonal antibody.

27. A method according to claim 26, wherein said monoclonal antibody specifically binds the CD4 antigen.

28. A method according to claim 1, wherein the step of contacting said test sample with said specific binding substance which specifically binds to said selected subset additionally includes contacting said test sample with an auxiliary specific binding substance which specifically binds to said specific binding substance which specifically binds to said selected subset, said auxiliary specific binding substance being affixed to a solid phase.

29. A method according to claim 21, wherein said test sample is contacted with a biotinylated antibody as the specific binding substance and with avidin as the auxiliary specific binding substance.

30. A method according to claim 29, wherein said biotin is attached to said antibody by a cleavable linkage.

31. A method according to claim 28, wherein said test sample is contacted with a first antibody as the specific binding substance and with a second antibody as the auxiliary specific binding substance.

32. A method according to claim 31, wherein said first antibody comprises a selected isotype and said second antibody binds selectively to a determinant of said isotype.

33. A method according to claim 31, wherein said first antibody comprises immunoglobulin obtained from a selected species and said second antibody binds selectively to a determinant of said immunoglobulin from said species.

34. A method for analyzing a subpopulation of cells, having at least one characteristic determinant, present within a mixed cell population, said subpopulation of cells including individual subsets of interest, each subset having at least one characteristic determinant, to determine the proportion of at least one selected subset of said subpopulation of cells, comprising: (i) selectively binding to a portion of cells of said mixed cell population a detectable reporter substance, either by a)

directly binding a specific binding substance which specifically binds to a determinant of said portion of cells and which is directly or indirectly conjugated to said detectable reporter substance or b) sequentially binding a first specific binding substance which specifically binds to a determinant of said portion of cells, and a second specific binding substance which specifically binds to said first specific binding substance and which is directly or indirectly conjugated to said detectable reporter substance, whereby said detectable reporter substance uniformly labels substantially all cells of said subpopulation; (ii) contacting a first sample of said mixed cell population with a first reagent comprising at least one specific binding substance which specifically binds to said at least one characteristic determinant of said cell subpopulation, resulting in the formation of a first complex of cells and uncomplexed cells; (iii) separating said first complex from said uncomplexed cells; (iv) following the completion of steps (i), (ii), and (iii), detecting the occurrence of said detectable reporter substance in said first complex; (v) contacting a second sample of said cell population from step (i), of equivalent volume and cell concentration to said first sample, with a second reagent comprising a specific binding substance which specifically binds to said at least one characteristic determinant of said selected subset of interest included in said subpopulation of cells, resulting in the formation of a second complex of cells and uncomplexed cells; (vi) separating said second complex from said uncomplexed cells; (vii) detecting the occurrence of said detectable reporter substance in said second complex; and (viii) determining the proportion of said selected subset of interest in said cell subpopulation by quantitating the amount of said detectable reporter substance associated with said second complex relative to the amount of said detectable reporter substance associated with said first complex.

35. A method according to claim 34, which further comprises: (ix) contacting one or more additional samples of said mixed cell population from step (i), each sample being of equivalent volume and cell concentration to said first sample, with additional reagents, each said additional reagent comprising a specific binding substance which binds specifically to a characteristic determinant of an additional subset of interest included in said subpopulation of cells, resulting in the formation of additional complexes of cells and uncomplexed cells; (x) separating each of said additional complexes from said uncomplexed cells; (xi) detecting the occurrence of said detectable reporter substance in each of said additional complexes; and (xii) determining the proportion of each of said additional subsets in said cell population by quantitating the amount of reporter substance associated with each of said additional complexes relative to the amount of reporter substance associated with said first complex.

36. A method according to claim 35 which includes contacting at least one of said additional samples with an auxiliary specific binding substance which specifically binds to said additional reagent with which said additional sample is contacted, said auxiliary specific binding substance being affixed to a solid phase.

37. A method according to claim 34, wherein said first reagent comprises a mixture of specific binding substances, each said binding substance specifically binding to a characteristic determinant of individual subsets of interest comprising said subpopulation, whereby substantially all cells of said subpopulation are bound to one or another of said binding substances.

38. A method according to claim 34, wherein said subpopulation comprises

lymphocytes and said subsets comprise lymphocytes characterized by selected functions or stages of differentiation.

39. A method according to claim 34, wherein said subpopulation comprises T-lymphocytes and said subsets are selected from the group consisting of helper T lymphocytes and suppressor/cytotoxic T lymphocytes.

40. A method according to claim 34, wherein said subpopulation comprises leukocytes and said subsets are selected from the group consisting of lymphocytes, monocytes and granulocytes.

41. A method according to claim 34, wherein said detectable reporter substance is removed from said complex prior to detecting its presence or quantity.

42. A method according to claim 34, wherein said specific binding substance, which specifically binds to a determinant of said portion of cells, comprises at least one antibody.

43. A method according to claim 42, wherein said reporter substance comprises an enzyme.

44. A method according to claim 43, wherein said enzyme is beta-galactosidase.

45. A method according to claim 42, wherein said reporter substance is attached to one member of a specific binding pair, said antibody is attached to the other member of said specific binding pair, and said selective binding is achieved by the combined effect of immunological binding and binding between the members of said specific binding pair.

46. A method according to claim 45, wherein said specific binding pair comprises avidin and biotin.

47. A method according to claim 46, wherein said biotin is attached to said antibody by a cleavable linkage.

48. A method according to claim 42, wherein said at least one antibody comprises a monoclonal antibody.

49. A method according to claim 48, wherein said monoclonal antibody specifically binds to CD45 antigen.

50. A method according to claim 49 wherein the amount of said monoclonal antibody bound per cell to lymphocytes is at least 5 time greater than the amount bound per cell to monocytes.

51. A method according to claim 48, wherein said at least one antibody comprises a monoclonal antibody that specifically binds T lymphocytes.

52. A method according to claim 57, wherein said at least one antibody comprises a monoclonal antibody which specifically binds to an antigen selected from the group consisting of CD2, CD3, CD5, CD7 or any combination of said monoclonal antibodies.

53. A method according to claim 34, wherein the step of selectively binding said detectable reporter substance to said subpopulation of cells comprises sequentially binding to the surface of the cells, a first antibody which specifically binds to at least one determinant which is common to the cells of said portion of cells, and a second

antibody which specifically binds to a determinant of said first antibody, and which is conjugated to said reporter substance.

54. A method according to claim 53, wherein said first antibody comprises a selected isotype and said second antibody specifically binds to a determinant of said isotype.

55. A method according to claim 53 wherein said first antibody comprise immunoglobulin obtained from a selected species and said second antibody specifically binds to a determinant of immunoglobulin from said selected species.

56. A method according to claim 34, wherein said specific binding substance of said first reagent and said specific binding substance of said second reagent are each affixed to a solid phase.

57. A method according to claim 56 wherein said second sample is contacted in a sample container with said specific binding substance which specifically binds said selected subset and wherein said specific binding substance which specifically binds said selected subset comprises antibody affixed to a surface of said container in contact with said second sample.

58. A method according to claim 34, wherein said specific binding substance of said first reagent and said specific binding substance of said second reagent are each affixed to magnetic or paramagnetic particles, and said first complex including said subpopulation of interest and said second complex including said selected subset are magnetically separated from said samples.

59. A method according to claim 34, wherein the specific binding substance of each of said first reagent and said second reagent comprises at least one antibody.

60. A method according to claim 34, wherein the specific binding substance of each of said first reagent and said second reagent comprises at least one monoclonal antibody.

61. A method according to claim 60, wherein said monoclonal antibody of said second reagent specifically binds the CD4 antigen.

62. A method according to claim 34 which includes contacting said first sample or said second sample with an auxiliary specific binding substance capable of interacting selectively with said first reagent or said second reagent, respectively, said auxiliary specific binding substance being affixed to a solid phase.

63. A method according to claim 62 wherein said first sample or said second sample is contacted with antibody as the auxiliary specific binding substance.

64. A method according to claim 62 wherein said first reagent or said second reagent comprises antibodies of a selected isotype, and said auxiliary specific binding substance comprises an antibody which binds selectively to a determinant of said isotype.

65. A method according to claim 62 wherein said first reagent or said second reagent comprises immunoglobulin obtained from a selected species, and said auxiliary specific binding substances comprise an antibody which binds selectively to a determinant of said immunoglobulin

from said species.

66. A method according to claim 62 wherein said first sample or said second sample is contacted with a biotinylated antibody as the first or second specific binding substance, respectively, and with avidin as the auxiliary specific binding substance.

67. A method according to claim 66 wherein said biotin is attached to said antibody by a cleavable linkage.

68. A test kit for determining the presence or quantity of one or more selected subsets of a subpopulation of cells in a test sample, said subset having at least one characteristic determinant, said test kit comprising: (i) a conjugate comprising a detectable reporter substance and a specific binding substance that specifically binds to at least one characteristic determinant of said subpopulation, said conjugate effecting uniform labelling of substantially all cells of said subpopulation by said detectable reporter substance, such that a consistent amount of reporter substance is distributed in the subset of interest; and (ii) a reagent comprising at least one specific binding substance affixed to a solid phase, said binding substance being capable of binding to at least one characteristic determinant of one of said subsets.

69. A test kit according to claim 68 which further comprises: i) a medium for uniformly labelling cells of said subpopulation with said reporter substance; ii) at least one pre-determined standard for determining the presence or quantity of said subsets in said test sample; and iii) reagent for detecting said reporter substance.

70. A test kit according to claim 68 wherein said solid phase comprises magnetic or paramagnetic material.

L42 ANSWER 21 OF 23 USPATFULL
96:50762 Therapeutic and diagnostic methods using total leukocyte surface antigens.
Rittershaus, Charles W., Malden, MA, United States
T Cell Diagnostics, Inc., St. Cambridge, MA, United States (U.S. corporation)
US 5525461 19960611
WO 9208981 19920529
APPLICATION: US 1993-50387 19930506 (8)
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to the measurement of total leukocyte antigens, or fragments thereof, and the use of such measurements to enumerate cells, especially in whole blood. The term "total" leukocyte antigen used herein refers to the total amount of a leukocyte antigen in a sample, including that present in membrane and intracellular compartments and extracellular soluble compartments. Measurements of a total leukocyte antigen can be used to type cells, detect or diagnose disease or to monitor disease therapy. In a further embodiment, the invention relates to the measurement of both the amount of total leukocyte antigen and the amount of the soluble form of the leukocyte antigen and a comparison of the measured levels.

CLM What is claimed is:
1. A method for determining the total amount of a leukocyte marker in an original sample suspected of having the leukocyte marker, which original

sample contains cells and a biological fluid in which cells are obtained, comprising: (a) adding a non-ionic detergent to the original sample to form a detergent treated sample in an amount sufficient to release any intracellular and membrane bound markers; (b) allowing the detergent treated sample to lyse the cells therein; (c) diluting the detergent treated sample by at least two fold; and (d) determining the amount of leukocyte marker in the detergent treated sample and calculating the total amount of leukocyte marker in the original sample therefrom.

2. The method according to claim 1 in which the non-ionic detergent comprises more than one non-ionic detergent.

3. The method according to claim 1 or claim 2 in which the non-ionic detergent is selected from the group consisting of TRITON.RTM." X-100, NONIDET.RTM." P-40, Tween-20 and CHAPS.

4. The method according to claim 1 in which the nonionic detergent comprises "TRITON.RTM." X-100 at a concentration of 1% to 2% and "NONIDET.RTM." P-40 at a concentration of 1% to 2%, which concentrations of "TRITON.RTM." X-100 and "NONIDET.RTM." are their respective concentrations in the detergent treated sample.

5. The method according to claim 1 in which the concentration of non-ionic detergent in the detergent-treated sample is a virus-inactivating concentration of non-ionic detergent.

6. The method according to claim 1 in which the non-ionic detergent is allowed to lyse the cells for at least about 1 minute.

7. The method according to claim 1 in which the detergent-treated sample is stored at about -20.degree. C. or less after step (b).

8. The method according to claim 7 in which the detergent-treated sample is stored at about -70.degree. C. or less.

9. The method according to claim 1 in which the original sample is selected from the group consisting of whole blood, blood cells, saliva, urine, synovial fluid, pleural effusions, tumor and tissue infiltrates, amniotic fluid, spinal fluid, cranial fluid, tissue, and tissue culture fluid.

10. The method according to claim 9 in which the sample is whole blood.

11. The method according to claim 1 in which the determination of step(d) is accomplished by an immunoassay selected from the group consisting of radio immunoassay, enzyme-linked immunoassay, "sandwich" immunoassay, immunoradiometric assay, fluorescence immunoassay and protein A immunoassay.

12. The method according to claim 11 in which the immunoassay detection means is a "sandwich" immunoassay.

13. The method according to claim 12 in which the sandwich immunoassay employs a polyclonal antibody.

14. The method according to claim 13 in which the sandwich immunoassay further employs a monoclonal antibody.

15. The method according to claim 1 in which the leukocyte marker is selected from the group consisting of CD4, CD8 and T cell

antigen receptor.

16. The method according to claim 14 or 15 in which the leukocyte marker is selected from the group consisting Of CD4, CD8 and T cell antigen receptor.

17. The method according to claim 1 in which the leukocyte marker is CD4 and in which the determination of step (d) is accomplished by a sandwich immunoassay employing a first antibody 8F4, deposited with the ATCC and assigned accession no. HB 9843, and a second antibody R2B7, deposited with the ATCC and assigned accession no. HB 9842.

18. The method according to claim 1 in which the leukocyte marker is CD8 and in which the determination of step (d) is accomplished by a sandwich immunoassay employing a first antibody 4C9, deposited with the ATCC and assigned accession no. HB 9340, and a second antibody 5F4/7B12, deposited with the ATCC and assigned accession no. HB 9342.

19. The method according to claim 1 in which the leukocyte marker is a .beta. T cell antigen receptor, and in which the determination of step (d) is accomplished by a sandwich immunoassay employing a first antibody selected from the group consisting of V.beta.S-specific antibody W112, deposited with the ATCC and assigned accession no. HB9927, and C.beta.-specific antibody W4F.5b, deposited with the ATCC and assigned accession no. HB 9282, and a second antibody 8A3.31 (.beta.F1), deposited with the ATCC and assigned accession no. HB 9283.

20. The method according to claim 1 in which the leukocyte marker is a .delta. T cell antigen receptor, and in which the determination of step (d) is accomplished by a sandwich immunoassay employing a first antibody TCF.delta.1, deposited with the ATCC and assigned accession no. HB 9772, and a second antibody .delta.TCS1, deposited with the ATCC and assigned accession no. HB 9578.

21. A method for determining the total amount of a leukocyte marker in an original sample suspected of having the leukocyte marker which original sample contains cells and the biological fluid in which the cells are obtained, comprising: (a) adding a non-ionic detergent to the original sample to form a detergent-treated sample such that the final concentration of the non-ionic detergent in the detergent treated sample is 2% to 4%: (b) allowing the detergent to lyse the cells therein; (c) diluting the detergent treated sample by at least 2-fold; and (d) determining the amount of leukocyte marker in the detergent treated sample and calculating the total amount of leukocyte marker in the original sample therefrom.

22. A method for diagnosing, detecting or staging a disease comprising detecting in a sample from a patient suspected of having the disease the total amount of a leukocyte marker according to the method of claim 1, 2, 4, 12 or 21 and comparing the amount so detected to the amount of the total leukocyte marker in normal individuals, a difference in amounts indicating a disease state.

23. The method according to claim 22 in which the leukocyte marker is selected from the group consisting of CD4, CD8 and T cell antigen receptor.

24. The method according to claim 23 in which the leukocyte marker is CD4 and the disease is selected from the group consisting of acquired immunodeficiency syndrome (AIDS), AIDS related complex, and CD4.sup.+ leukemia or lymphoma.

25. The method according to claim 23 in which the leukocyte marker is CD8 and the disease is selected from the group consisting of AIDS, transplantation rejection, rheumatoid arthritis, and infectious disease.

26. A method for diagnosing, detecting or staging a disease in a patient suspected of having the disease comprising: (a) measuring in an original sample from the patient the total amount of at least two leukocyte markers according to the method of claim 1, 2, 4, 12 or 21; (b) determining the ratio or ratios of the amounts of the leukocyte markers measured in step (a); and (c) comparing the ratio or ratios of the leukocyte markers determined in step (b) to the same ratio or ratios in a normal individual, a difference in the ratios indicating a disease state.

27. The method of claim 26 in which the leukocyte markers are CD4 and CD8 and the disease is AIDS.

28. A method for monitoring the effect of a therapeutic treatment for a disease comprising detecting in a sample from a patient undergoing therapeutic treatment for the disease the total amount of a leukocyte marker according to the method of claim 1, 2, 4, 12 or 21 and comparing the amount so detected to the amount of the total leukocyte marker in normal individuals, a difference in amounts indicating a change in the disease state.

29. The method according to claim 28 in which the leukocyte marker is selected from the group consisting of CD4, CD8 and T cell antigen receptor.

30. The method according to claim 29 in which the leukocyte marker is CD4 and the disease is selected from the group consisting of acquired immunodeficiency syndrome (AIDS), AIDS related complex, and CD4.sup.+ leukemia or lymphoma.

31. The method according to claim 29 in which the leukocyte marker is CD8 and the disease is selected from the group consisting of AIDS, transplantation rejection, rheumatoid arthritis, and infectious disease.

32. A method for monitoring the effect of a therapeutic treatment for a disease in a patient undergoing therapeutic treatment for the disease comprising: (a) measuring in a sample from the patient the total amount of at least two leukocyte markers according to the method of claim 1, 2, 4, 12 or 21; (b) determining the ratio or ratios of the amounts of the leukocyte markers measured in step (a); and (c) comparing the ratio or ratios of the leukocyte markers determined in step (b) to the same ratio or ratios in a normal individual, a difference in the ratios indicating a change in the disease state.

33. The method of claim 32 in which the leukocyte markers are CD4 and CD8 and the disease is AIDS, and in which an increase in the ratio of CD4 to CD8 positive leukocytes in the patient relative to the patient at an earlier time is indicative improvement of the disease.

34. A method for enumerating the total number of leukocytes that are positive for a CD4 leukocyte marker in an original sample, which original sample contains cells and a biological fluid in which cells are obtained, comprising: (a) adding a non-ionic detergent to the original sample to form a detergent treated sample such that the final concentration of the non-ionic detergent in the detergent treated sample

is 2% to 4%; (b) allowing the detergent to lyse the cells therein; (c) diluting the detergent treated sample by at least two fold; and (d) detecting the total amount of the CD4 leukocyte marker in the detergent treated sample; and (e) calculating the total amount of leukocytes positive for the CD4 leukocyte marker from the total amount of CD4 leukocyte marker detected in step (d).

35. The method according to claim 34 in which the non-ionic detergent comprises more than one non-ionic detergent.

36. The method according to claim 34 or 35 in which the non-ionic detergent is selected from the group consisting of "TRITON.RTM." X-100, "NONIDET.RTM." P-40, Tween-20 and CHAPS.

37. The method according to claim 36 in which the non-ionic detergent comprises "TRITON.RTM." X-100 at a concentration of 1% to 2% and "NONIDET.RTM." P-40 at a concentration of 2%, which concentrations of "TRITON.RTM." X-100 and "NONIDET.RTM." P-40 are their respective concentrations in the detergent-treated sample.

38. The method according to claim 37 in which the non-ionic detergent is allowed to lyse the cells for at least about 1 minute.

39. The method according to claim 38 in which the detergent-treated sample is stored at about -20.degree. C. or less after step (b).

40. The method according to claim 39 in which the original sample is selected from the group consisting of whole blood, blood cells, saliva, urine, synovial fluid, pleural effusions, tumor and tissue infiltrates, amniotic fluid, spinal fluid, cranial fluid, tissue, and tissue culture fluid.

41. The method according to claim 34 in which the original sample is whole blood.

42. The method according to claim 35 in which the original sample is whole blood.

43. The method according to claim 36 in which the original sample is whole blood.

44. The method according to claim 37 in which the original sample is whole blood.

45. The method according to claim 38 in which the original sample is whole blood.

46. The method according to claim 39 in which the original sample is whole blood.

47. The method according to claim 40 in which the original sample is whole blood.

48. The method according to claim 40 in which the determination of step (d) is accomplished by an immunoassay selected from the group consisting of radioimmunoassay, enzyme-linked immunoassay, "sandwich" immunoassay, immunoradiometric assay, fluorescence immunoassay and protein A immunoassay.

49. The method according to claim 34 in which the immunoassay is a "sandwich" immunoassay.

50. The method according to claim 42 in which the sandwich immunoassay employs a polyclonal antibody.

51. The method according to claim 42 in which the sandwich immunoassay further employs a monoclonal antibody.

52. The method according to claim 44 in which the immunoassay detection means is a sandwich immunoassay employing a first antibody 8F4, deposited with the ATCC and assigned accession no. HB 9843, and a second antibody R2B7, deposited with the ATCC and assigned accession no. HB 9842.

53. The method according to claim 34 further comprising: (i) separately measuring the amount of soluble leukocyte marker in the original sample; (ii) subtracting the amount of soluble leukocyte marker in the original sample from the amount of total leukocyte marker in the original sample determined in step (d); and, (iii) in step (e) calculating the number of cells positive for the leukocyte marker in the original sample from the remainder in step (ii).

54. A method for enumerating the total number of leukocytes that are positive for a CD8 leukocyte marker in an original sample, which original sample contains cells and the biological fluid in which the cells are obtained comprising: (a) adding a non-ionic detergent to the original sample to form a detergent treated sample such that the final concentration of the non-ionic detergent in the detergent treated sample is 2% to 4%; (b) allowing the detergent to lyse the cells therein; (c) diluting the detergent treated sample by at least two fold; and (d) detecting the total amount of the CD8 leukocyte marker in the detergent treated sample; and (e) calculating the number total amount of leukocytes positive for the CD8 leukocyte marker from the total amount of CD8 leukocyte marker detected in step (d).

55. The method according to claim 54 in which the non-ionic detergent comprises more than one non-ionic detergent.

56. The method according to claim 54 or 55 in which the non-ionic detergent is selected from the group consisting of "TRITON.RTM." X-100, "NONIDET.RTM." P-40, Tween-20 and CHAPS.

57. The method according to claim 56 in which the non-ionic detergent comprises "TRITON.RTM." X-100 at a concentration of 1% to 2% and "NONIDET.RTM." P-40 at a concentration of 1% to 2%, which concentrations of "TRITON.RTM." X-100 and "NONIDET.RTM." P-40 are their respective concentrations in the detergent-treated sample.

58. The method according to claim 57 in which the non-ionic detergent is allowed to lyse the cells for at least about 1 minute.

59. The method according to claim 58 in which the detergent-treated sample is stored at about -20.degree. C. or less after step (b).

60. The method according to claim 59 in which the original sample is selected from the group consisting of whole blood, blood cells, saliva, urine, synovial fluid, pleural effusions, tumor and tissue infiltrates, amniotic fluid, spinal fluid, cranial fluid, tissue, and tissue culture fluid.

61. The method according to claim 54 in which the original sample is whole blood.

62. The method according to claim 55 in which the original sample is whole blood.

63. The method according to claim 56 in which the original sample is whole blood.

64. The method according to claim 57 in which the original sample is whole blood.

65. The method according to claim 58 in which the original sample is whole blood.

66. The method according to claim 59 in which the original sample is whole blood.

67. The method according to claim 60 in which the original sample is whole blood.

68. The method according to claim 60 in which the determination of step (d) is accomplished by an immunoassay selected from the group consisting of radioimmunoassay, enzyme-linked immunoassay, "sandwich" immunoassay, immunoradiometric assay, fluorescence immunoassay and protein A immunoassay.

69. The method according to claim 68 in which the immunoassay is a "sandwich" immunoassay.

70. The method according to claim 69 in which the sandwich immunoassay employs a polyclonal antibody.

71. The method according to claim 70 in which the sandwich immunoassay further employs a monoclonal antibody.

72. The method according to claim 71 in which the immunoassay is a sandwich immunoassay employing a first antibody 4C9, deposited with the ATCC and assigned accession no. HB 9340, and a second antibody 5F4/7B12, deposited with the ATCC and assigned accession no. HB 9342.

73. The method according to claim 54 further comprising: (i) separately measuring the amount of soluble leukocyte marker in the original sample; (ii) subtracting the amount of soluble leukocyte marker in the original sample from the amount of total leukocyte marker in the original sample determined in step (d); and (iii) in step (e) calculating the number of cells positive for the leukocyte marker in the original sample from the remainder in step (ii).

74. A method for enumerating the total number of leukocytes that are positive for a T cell antigen receptor leukocyte marker in an original sample, which original sample contains cells and the biological fluid in which the cells are obtained, comprising: (a) adding a non-ionic detergent to the original sample to form a detergent treated sample such that the final concentration of the non-ionic detergent in the detergent treated sample is 2% to 4%; (b) allowing the detergent to lyse the cells therein; (c) diluting the detergent treated sample by at least two fold; and (d) detecting the total amount of the T cell antigen receptor leukocyte marker in the detergent treated sample; and (e) calculating the total amount of leukocytes positive for the T cell antigen receptor leukocyte marker from the total amount of the T cell antigen receptor leukocyte marker detected in step (d).

75. The method according to claim 74 in which the non-ionic detergent comprises more than one non-ionic detergent.

76. The method according to claim 74 or 75 in which the non-ionic detergent is selected from the group consisting of "TRITON.RTM." X-100, "NONIDET.RTM." P-40, Tween-20 and CHAPS.

77. The method according to claim 76 in which the non-ionic detergent comprises "TRITON.RTM." X-100 at a concentration of 1% to 2% and "NONIDET.RTM."P-40 at a concentration of 1% to 2%, which concentrations of "TRITON.RTM." X-100 and "NONIDET.RTM."P-40 are their respective concentrations in the detergent-treated sample.

78. The method according to claim 77 in which the non-ionic detergent is allowed to lyse the cells for at least about 1 minute.

79. The method according to claim 78 in which the detergent-treated sample is stored at about -20.degree. C. or less after step (b).

80. The method according to claim 79 in which the original sample is selected from the group consisting of whole blood, blood cells, saliva, urine, synovial fluid, pleural effusions, tumor and tissue infiltrates, amniotic fluid, spinal fluid, cranial fluid, tissue, and tissue culture fluid.

81. The method according to claim 74 in which the original sample is whole blood.

82. The method according to claim 75 in which the original sample is whole blood.

83. The method according to claim 76 in which the original sample is whole blood.

84. The method according to claim 77 in which the original sample is whole blood.

85. The method according to claim 78 in which the original sample is whole blood.

86. The method according to claim 79 in which the original sample is whole blood.

87. The method according to claim 80 in which the original sample is whole blood.

88. The method according to claim 80 in which the immunoassay is selected from the group consisting of radioimmunoassay, enzyme-linked immunoassay, "sandwich" immunoassay, immunoradiometric assay, fluorescence immunoassay and protein A immunoassay.

89. The method according to claim 88 in which the immunoassay is a "sandwich" immunoassay.

90. The method according to claim 89 in which the sandwich immunoassay employs a polyclonal antibody.

91. The method according to claim 89 in which the sandwich immunoassay further employs a monoclonal antibody.

92. The method according to claim 91 in which the T cell antigen

receptor is a .beta. T cell antigen receptor, and in which the immunoassay is a sandwich immunoassay employing a first antibody selected from the group consisting of V.beta.5-specific antibody W112, deposited with the ATCC and assigned accession no. HB 9927, and C.beta.-specific antibody W4F.5B, deposited with the ATCC and assigned accession no. HB 9832, and a second antibody 8A3.31 (.beta.F1), deposited with the ATCC and assigned accession no. HB 9283.

93. The method according to claim 91 in which the T cell antigen receptor is a .beta. T cell antigen receptor, and in which the immunoassay is a sandwich immunoassay employing a first antibody TCR.delta.1, deposited with the ATCC and assigned accession no. HB 9772, and a second antibody .delta.TCS1, deposited with the ATCC and assigned accession no. HB 9578.

94. The method according to claim 74 further comprising: (i) separately measuring the amount of soluble leukocyte marker in the original sample; (ii) subtracting the amount of soluble leukocyte marker in the original sample from the amount of total leukocyte marker in the original sample determined in step (d); and (iii) in step (e) calculating the number of cells positive for the leukocyte marker in the original sample from the remainder in step (ii).

95. A method for diagnosing, detecting or staging a disease in a patient suspected of having the disease comprising enumerating the number of leukocytes that are positive for a leukocyte marker in a sample from the patient according to the method of claim 34, 54 or 74 and comparing the number of leukocytes with the number of leukocytes found in a normal individual, a difference in the numbers indicating a disease state.

96. The method according to claim 95 in which the leukocyte marker is CD4 and the disease is selected from the group consisting of acquired immunodeficient syndrome (AIDS), AIDS related complex, and CD4.sup.+ leukemia or lymphoma.

97. The method according to claim 95 in which the leukocyte marker is CD8 and the disease is selected from the group consisting of AIDS, transplantation rejection, rheumatoid arthritis, and infectious disease.

98. A method of diagnosing, detecting or staging a disease in a patient suspected of having the disease comprising: (a) enumerating the number of leukocytes that are positive for at least two leukocyte markers in a sample from the patient according to the method of claim 34, 54 or 74, (b) determining the ratio or ratios of leukocytes positive for each of the leukocyte markers enumerated in step (a); and (c) comparing the ratio or ratios of leukocytes determined in step (b) to the ratio or ratios in a normal individual, a difference in the ratios indicating a disease state.

99. The method of claim 98 in which the leukocyte markers are CD4 and CD8 and the disease is AIDS.

100. A method for monitoring the effect of a therapeutic treatment for a disease in a patient undergoing therapeutic treatment for the disease comprising enumerating the number of leukocytes that are positive for a leukocyte marker in a sample from the patient according to the method of claim 34, 54, or 74 and comparing the approximate number of leukocytes with the number of the leukocytes found in normal individual, a difference in the number indicating a change in the disease state.

101. The method according to claim 100 in which the leukocyte marker is

CD4 and the disease is selected from the group consisting of acquired immunodeficiency syndrome (AIDS), AIDS related complex, and CD4.sup.+ leukemia or lymphoma.

102. The method according to claim 100 in which the leukocyte marker is CD8 and the disease is selected from the group consisting of AIDS, transplantation rejection, rheumatoid arthritis, and infectious disease.

103. A method of monitoring the effect of a therapeutic treatment for a disease in a patient undergoing therapeutic treatment for the disease comprising: (a) enumerating the number of leukocytes that are positive for at least two leukocyte markers in a sample from the patient according to the method of claim 34, 59 or 74; (b) determining the ratio or ratios of leukocytes positive for each of the leukocyte markers enumerated in step (a); and (c) comparing the ratio or ratios of leukocytes determined in step (b) to the ratio or ratios in the patient at an earlier time difference in the ratios indicating a change in the disease state.

104. The method of claim 103 in which the leukocyte markers are CD4 and CD8 and the disease is AIDS, and in which an increase in the ratio of CD4 to CD8 positive leukocytes in the patient relative to the patient at an earlier time is indicative of improvement of the disease.

105. A kit for enumerating the number of leukocytes that are positive for a CD4 leukocyte marker in a sample comprising: (a) a concentrated non-ionic detergent solution comprising 9% "TRITON.RTM." X-100 and 6% "NONIDET.RTM." P-40 in distilled water; and (b) an immunoassay employing a first antibody 8F4, deposited with the ATCC and assigned accession no. HB 9843, and a second antibody R2B7, deposited with the ATCC and assigned accession no HB 9853.

106. A kit for enumerating the number of leukocytes that are positive for a CD8 leukocyte marker in a sample comprising: (a) a concentrated non-ionic detergent solution comprising 9% "TRITON.RTM." X-100 and 6% "NONIDET.RTM." P-40 in distilled water; and (b) an immunoassay employing a first antibody 4C9, deposited with the ATCC and assigned accession no. HB 9340, and a second antibody 5F4/7B12, deposited with the ATCC and assigned accession no. HB 9353.

107. A kit for enumerating the number of leukocytes that are positive for a .beta. T cell antigen receptor leukocyte marker in a sample comprising: (a) a concentrated non-ionic detergent solution comprising 9% "TRITON.RTM." X-100 and 6% "NONIDET.RTM." P-40 in distilled water; and (b) an immunoassay employing a first antibody selected from the group consisting of V.beta.5-specific antibody W112, deposited with the ATCC and assigned accession no. HB 9927, and C.beta.-specific antibody W4F.5B, deposited with the ATCC and assigned accession no. HB 9832, and a second antibody 8A3.31 (.beta.F1), deposited with the ATCC and assigned accession no. HB 9283.

108. A kit for enumerating the number of leukocytes that are positive for a delta T cell antigen receptor leukocyte marker in a sample comprising: (a) a concentrated nonionic detergent solution comprising 9% "TRITON.RTM." X-100 and 6% "NONIDET.RTM." P-40 in distilled water; and (b) an immunoassay employing a first antibody TCR.delta.1, deposited with the ATCC and assigned accession no. HB 9772, and a second antibody .delta.TCS1, deposited with the ATCC and assigned accession no. HB 9578.

L42 ANSWER 20 OF 23 USPATFULL

97:7798 Cell fixative and method of analyzing virally infected cells.
Connelly, Mark C., Doylestown, PA, United States
Chakraborty, Utpal R., Flemington, NJ, United States
Brooks, Jr., Houston G., Somerset, NJ, United States
Ortho Diagnostic Systems Inc., Raritan, NJ, United States (U.S. corporation)
US 5597688 19970128
APPLICATION: US 1995-467799 19950606 (8)
DOCUMENT TYPE: Utility; Granted.

AB The present invention provides a novel cellular fixative composition and method of cellular fixing and cellular analysis wherein cells may be fixed for further analysis without destroying the cell's surface markers, cellular morphology, and the cell's light scattering properties. Treatment of cells with the fixative as described herein also allows antibodies or .other desired components to enter the cell through the cellular membrane, without allowing important contents of the cell to escape. As an added benefit, the fixative composition of the invention may also kill or at least inactivate or reduce the activity of deadly virus contained in the cell. In its broadest aspect, the presently claimed fixative composition comprises at least three components, and in more preferred embodiments, four components. In a particularly preferred embodiment, the fixative composition described herein comprises at least one compound suitable for increasing the permeability of a cellular membrane, at least one compound that facilitates transportation of components across cellular membranes, at least one detergent, and at least one compound having the structure:

R.sub.1 R.sub.2 R.sub.3 R.sub.4 R.sub.5 --Ar--X

CLM What is claimed is:
1. A method for monitoring human immunodeficiency virus (HIV) infection in a patient so infected, comprising the steps of: a) contacting a sample of whole blood from said patient with a fixative composition in an amount and for a period of time effective to fix white blood cells present in said whole blood, without substantially destroying the ability of white blood cell antigens and viral components to bind ligands, wherein said fixative composition comprises: i) a first fixative compound selected from the group consisting of 2,4-dinitrobenzene sulfonic acid, 2,4-dinitrobenzoic acid, 2,4-dinitrophenol, and a combination of two or more of these; ii) a second fixative compound which is methanol-free, high-grade formaldehyde in a concentration ranging from about 0.1% to about 4%; iii) dimethylsulfoxide in a concentration of about 1% (v/v) to about 20% (v/v); and iv) a polyoxyethylene sorbitan surfactant in a concentration of about 0.001% to about 0.2% (w/v); b) isolating white blood cells present in said sample; c) either concurrently with the contact Step in a) or thereafter, contacting the cells so fixed with at least one antibody to a white blood cell antigen and at least one binding ligand that binds to at least one component from HIV; d) examining the scattering and fluorescent properties of said cells so fixed with a flow cytometer; and e) comparing the results of said examination to data obtained in the same manner from patients at various stages of HIV infection.

2. The method of claim 1 wherein said first fixative compound is 2,4-dinitrobenzene sulfonic acid.

3. The method of claim 1 wherein said at least one binding ligand that binds to said component from HIV is anti-p24 antibody.

4. The method of claim 1 wherein said at least one antibody to a white blood cell antigen is an anti-CD4 monoclonal antibody.

5. The method of claim 4 wherein said anti-CD4 monoclonal antibody is labelled with phycoerythrin and said anti-p24 antibody is labelled with FITC.

6. A reagent kit for monitoring HIV load in HIV -infected white blood cells, comprising: a) a fixative composition which comprises: i) a first fixative compound selected from the group consisting of 2,4-dinitrobenzene sulfonic acid, 2,4-dinitrobenzoic acid, 2,4-dinitrophenol, and a combination of two or more of these; ii) a second fixative compound which is methanol-free, high-grade formaldehyde in a concentration ranging from about 0.1% to about 4%; iii) dimethylsulfoxide in a concentration of about 1% (v/v) to about 20% (v/v); and iv) a polyoxyethylene sorbitan surfactant in a concentration of about 0.001% to about 0.2% (w/v); and b) a binding ligand that binds to an intracellular antigen from HIV.

7. The reagent kit of claim 6 further comprising at least one antibody to a white blood cell surface antigen.

8. The reagent kit of claim 7 wherein at least one of said antibodies to a white blood cell surface antigen is a monoclonal antibody to CD4 positive T cells.

9. The reagent kit of claim 8 further comprising at least one monoclonal antibody to monocytes.

L42 ANSWER 18 OF 23 USPATFULL
97:38380 One step method for detection and enumeration of absolute counts of one more cell populations in a sample.
Ward, Anthony J., San Ramon, CA, United States
Mercolino, Thomas J., Pleasanton, CA, United States
Recktenwald, Diether J., Cupertino, CA, United States
Becton Dickinson and Company, Franklin Lakes, NJ, United States (U.S. corporation)
US 5627037 19970506
APPLICATION: US 1994-287759 19940809 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention comprises a one step method for the detection and enumeration of absolute counts of one or more cell populations in a blood sample. The method employs a reagent comprising a mixture of one or more cell markers, a fluorescent microparticle and a fixative. The reagent may be combined with unlysed whole blood and analyzed by means of flow cytometry.

CLM What is claimed is:
1. A method for determining the absolute counts of CD4.sup.+ lymphocytes within a whole blood sample by means of flow cytometry comprising the steps of: (a) adding the sample to a tube containing a diluent comprising an anti-CD4 monoclonal antibody bound to phycoerythrin, paraformaldehyde and a known concentration of a 2 .mu.m autofluorescent coumarin microparticle; (b) setting a fluorescence trigger in the second fluorescence channel to include at least 99% of all microparticles and CD4.sup.+ lymphocytes; (c) setting fluorescence gates in the first and second fluorescence channels to distinguish between the

phycoerythrin fluorescence associated with the labelled CD4 .sup.+ lymphocytes and the autofluorescence of the microparticle; (d) counting the number of CD4.sup.+ lymphocytes and microparticles from step (c); and (e) calculating the number of CD4.sup.+ lymphocytes per microparticle from step (d) and multiplying by the concentration of microparticles resulting in the absolute count of CD4.sup.+ lymphocytes per unit volume.

2. A method of staining cells in a sample containing said cells with one or more immunofluorescence markers, said immunofluorescence markers comprising a monoclonal antibody bound to a fluorochrome, wherein the sample is added directly to a tube containing a diluent, said diluent comprising said one or more immunofluorescence markers and a fixative.

3. The method of claim 2 wherein the fluorochromes are selected from the group consisting of phycobiliproteins, fluorescein derivatives, rhodamine, pthalocyanine derivatives, peridinin chlorophyll complex and coumarin derivatives.

4. The method of claim 3 wherein the fluorochrome is a phycobiliprotein.

5. The method of claim 4 wherein the phycobiliprotein is phycoerythrin.

6. The method of claim 2 wherein the fixative comprises the group consisting of paraformaldehyde and formaldehyde.

7. The method of claim 2 the concentration of the immunofluorescence marker is between 0.02 .mu.g/ml and 20 .mu.g/ml.

8. The method of claim 2 wherein the diluent further comprises a fluorescent microparticle.

9. A solution for staining cells comprising a diluent, said diluent comprising one or more immunofluorescence markers and a fixative.

10. The solution of claim 9 wherein the diluent further comprises a fluorescent microparticle.

11. The solution of claim 9 wherein the fixative is paraformaldehyde.

12. A solution for staining unlysed whole blood comprising a diluent, said diluent comprising a fluorescently labelled monoclonal antibody, a fixative and a fluorescent microparticle.

13. The solution of claim 12 wherein the antibody is an anti-CD4 monoclonal antibody.

14. The solution of claim 12 wherein the fluorescent label is phycoerythrin.

15. The solution of claim 12 wherein the fixative is paraformaldehyde.

16. The solution of claim 12 wherein the microparticle is an autofluorescent 2 .mu.m coumarin bead.

17. A solution for staining CD4.sup.+ lymphocytes in an unlysed whole blood sample wherein the solution comprises an anti-CD4 monoclonal antibody bound to phycoerythrin, paraformaldehyde and an autofluorescent 2 .mu.m coumarin bead.

L42 ANSWER 17 OF 23 USPATFULL
97:73455 Cell enumeration immunoassay.
Greene, Richard Alfred, Westford, MA, United States
Kasila, Patricia Ann, Windham, NH, United States
Bobrow, Mark Norman, Lexington, MA, United States
E. I. Du Pont de Nemours and Company, Wilmington, DE, United States (U.S. corporation)
US 5658745 19970819
APPLICATION: US 1995-390598 19950217 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to a cell enumeration immunoassay which uses a calibrated standard, i.e., a substance which behaves like the sample under study and the concentration of which can be correlated to the concentration of the cells. This immunoassay is an efficient alternative to flow cytometry.

CLM What is claimed is:

1. A cell enumeration immunoassay for quantitating the number of cells in a subpopulation or a subset of the subpopulation of a total cell population in a sample which comprises: (a) contacting the sample simultaneously with a modified solid phase, a first labeled antibody specific for the subpopulation and a second detectably labeled antibody specific for the subpopulation or the subset of the subpopulation wherein the label on the first antibody is used for immobilization onto the modified solid phase, the label on the second antibody is used for detection and the label on the first antibody is different from the label on the second antibody and further wherein the first antibody and the second antibody can have the same or different specificities and bind to different sites on the cells in the subpopulation wherein when the first and the second antibodies have the same specificity then the cells in the subpopulation have more than one copy of an epitope to which the first and second antibody can bind; (b) contacting separately a calibrated standard which can be labeled or unlabeled with the modified solid phase and (i) no other reactants if the calibrated standard is doubly labeled with the same labels attached to the first antibody and the second antibody of step (a), (ii) the first and second labeled antibodies if the calibrated standard is unlabeled and capable of binding to the first and second antibodies, (iii) the first labeled antibody only if the calibrated standard is singly labeled with the same label as the second antibody and is capable of binding with the first antibody, or (iv) the second labeled antibody only if the calibrated standard is singly labeled with the same label as the first antibody and is capable of binding with the second antibody, provided that if the calibrated standard is a cell then it is labeled either singly or doubly with the same label or labels attached to the first and second antibodies wherein when the cell is singly labeled said cell already has the other of the first or the second labels; (c) measuring separately a signal generated by step (a) and a signal generated by step (b); and (d) quantitating the number of cells in the subpopulation or subset of the subpopulation in the sample by comparing the results from the measurement of signal generated by step (a) with the results obtained from the measurement of signal generated by step (b).

2. An immunoassay according to claim 1 wherein the cells to be quantitated are selected from the group consisting of CD4 .sup.+ T cells, CD8.sup.+ T cells, B-cells, activated B cells, activated T cells, and CMV-infected granulocytes, EBV infected B-cells, and HIV infected monocytes.

3. A T cell enumeration immunoassay for quantitating the number of T cells in a subset of a total T cell population in a sample which comprises: (a) contacting the sample simultaneously with a modified solid phase, a labeled anti-pan T cell antibody wherein said label is used for immobilization onto the modified solid phase and a detectably labeled anti-subset specific antibody wherein the label on the anti-subset specific antibody is used for detection and is different from the label on the anti-pan T cell antibody and further wherein the anti-pan T cell antibody and the anti-subset specific antibody have different specificities and bind to different sites on the cells in the subpopulation; (b) contacting separately a calibrated standard which can be labeled or unlabeled with the modified solid phase and (i) no other reactants if the calibrated standard is doubly labeled with the same labels attached to the anti-pan T cell antibody and the anti-subset specific antibody of step (a), (ii) the labeled anti-pan T cell antibody and the labeled anti-subset specific antibody if the calibrated standard is unlabeled and capable of binding to both antibodies, (iii) the labeled anti-pan T cell only if the calibrated standard is singly labeled with the same label as the anti-subset specific antibody and is capable of binding to the anti-pan T cell antibody, or (iv) the labeled anti-subset specific antibody only if the calibrated standard is singly labeled with the same label as the anti-pan T cell antibody and is capable of binding with the anti-subset specific antibody, provided that if the calibrated standard is a cell then it is labeled either singly or doubly with the same label or labels attached to the antibodies wherein when the cell is singly labeled said cell already has the other of the labeled anti-pan T cell or the labeled anti-subset specific antibody; (c) measuring separately a signal generated by step (a) and a signal generated by step (b); and (d) quantitating the number of T cells in the subset of the total T cell population in the sample by comparing the results from the measurement of signal generated by step (a) with the results obtained from the measurement of signal generated by step (b).

4. An immunoassay according to claim 3 wherein the cells to be quantitated in the subpopulation are CD4.sup.+ T lymphocytes, the labeled anti-pan T cell antibody is a biotinylated anti-CD3 monoclonal antibody, the detectably labeled anti-subset specific antibody is a fluorescein labeled anti-CD4 monoclonal antibody and the calibrated standard is a biotin and fluorescein labeled dextran.

5. An immunoassay according to claim 1 or 3 wherein the solid phase is modified with a first member of a non-immune binding pair.

6. An immunoassay according to claim 1 or 3 wherein the solid phase is modified with a first member of an immune binding pair.

7. An immunoassay according to claim 5 wherein the first antibody is labeled with the second member of a non-immune binding pair.

8. An immunoassay according to claim 6 wherein the first antibody is labeled with the second member of an immune binding pair.

9. An immunoassay according to claim 1 or 3 wherein the second antibody is detectably labeled with a member of a non-immune binding pair.

10. An immunoassay according to claim 1 or 3 wherein the second antibody is detectably labeled with a member of an immune binding pair.

11. An immunoassay according to claim 1 or 3 wherein at least one of the antibodies is monoclonal.

12. An immunoassay according to claim 1 or 3 wherein the calibrated standard is selected from the group consisting of dextran labeled with biotin and fluorescein, bovine serum albumin labeled with biotin and fluorescein and biotinylated fluorescein.

13. An immunoassay according to claim 1 or 3 wherein the product of step (a) is reacted with an inactivation reagent to inactivate endogenous peroxidases, fix the cells in the sample and inactivate any human immunodeficiency virus which might be present in the sample.

L5 ANSWER 2 OF 38 MEDLINE
2001021610 Document Number: 20435932. PubMed ID: 10979936. CD4+ T cell surface CCR5 density and virus load in persons infected with human immunodeficiency virus type 1. Marmor M; Krowka J; Goldberg J D. JOURNAL OF INFECTIOUS DISEASES, (2000 Oct) 182 (4) 1284-6. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

L5 ANSWER 20 OF 38 MEDLINE
91208752 Document Number: 91208752. PubMed ID: 1708316. Epitopes of human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins recognized by antibodies in the sera of HIV-1-infected individuals. Krowka J F ; Singh B; Stites D P; Maino V C; Narindray D; Hollander H; Jain S; Chen H; Blackwood L; Steimer K S. (Department of Laboratory Medicine, University of California, San Francisco 94143.) CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY, (1991 Apr) 59 (1) 53-64. Journal code: 0356637. ISSN: 0090-1229. Pub. country: United States. Language: English.

AB Sera from human immunodeficiency virus (HIV)-infected study subjects and controls were analyzed by enzyme-linked immunosorbent assay using 10 synthetic peptides to identify epitopes of HIV envelope glycoproteins (ENVgp) that were recognized by antibodies. Two epitopes of HIV ENVgp, ENVP466 (amino acids 466-481) and ENVP497 (amino acids 497-509), were recognized by antibodies in the sera of most HIV-infected individuals. The frequency of individuals with detectable serum antibodies to these two epitopes was not associated with the stage of HIV disease. Purified antibodies to ENV497 had only very weak neutralizing activity against infectious HIV. These data suggest that a particular dominant type of antibody response to HIV's ENVgp has minimal protective effects. These and other studies to identify and stimulate immune responses to selected epitopes of HIV antigens may be useful in the design of vaccines to prevent or treat HIV infections.

L43 ANSWER 7 OF 23 MEDLINE
96323171 Document Number: 96323171. PubMed ID: 8709277. Human immunodeficiency virus type 1 membrane fusion mediated by a laboratory-adapted strain and a primary isolate analyzed by resonance energy transfer. Litwin V; Nagashima K A; Ryder A M; Chang C H; Carver J M; Olson W C; Alizon M; Hasel K W; Maddon P J; Allaway G P. (Progenics Pharmaceuticals, Inc., Tarrytown, New York 10591, USA.) JOURNAL OF VIROLOGY, (1996 Sep) 70 (9) 6437-41. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Previous studies of human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein-mediated membrane fusion have focused on laboratory-adapted T-lymphotropic strains of the virus. The goal of this study was to characterize membrane fusion mediated by a primary HIV-1 isolate in comparison with a laboratory-adapted strain. To this end, a new fusion assay was developed on the basis of the principle of resonance energy transfer, using HeLa cells stably transfected with gp120/gp41 from the T-lymphotropic isolate HIV-1LA1 or the macrophage-tropic primary isolate HIV-1JR-FL. These cells fused with CD4+ target cell lines with a tropism mirroring that of infection by the two viruses. Of particular note, HeLa cells expressing HIV-1JR-FL gp120/gp41 fused only with PM1 cells, a clonal derivative of HUT 78, and not with other T-cell or macrophage cell lines. These results demonstrate that the envelope glycoproteins of these strains play a major role in mediating viral tropism. Despite significant differences exhibited by HIV-1JR-FL and HIV-1LAI in terms of tropism and sensitivity to neutralization by CD4-based proteins, the present study found that membrane fusion mediated by the envelope glycoproteins of these viruses had remarkably similar properties. In particular, the degree and

kinetics of membrane fusion were similar, fusion occurred at neutral pH and was dependent on the presence of divalent cations. Inhibition of HIV-1JR-FL envelope glycoprotein-mediated membrane fusion by soluble CD4 and CD4-IgG2 occurred at concentrations similar to those required to neutralize this virus. Interestingly, higher concentrations of these agents were required to inhibit HIV-1LAI envelope glycoprotein-mediated membrane fusion, in contrast to the greater sensitivity of HIV-1LAI virions to neutralization by soluble CD4 and CD4-IgG2. This finding suggests that the mechanisms of fusion inhibition and neutralization of HIV-1 are distinct.

L46 ANSWER 28 OF 33 MEDLINE
95272427 Document Number: 95272427. PubMed ID: 7752929. Fluorescence resonance energy transfer. Selvin P R. (Calvin Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley 94720, USA.) METHODS IN ENZYMOLOGY, (1995) 246 300-34. Journal code: 0212271. ISSN: 0076-6879. Pub. country: United States. Language: English.

L48 ANSWER 19 OF 31 MEDLINE
96050774 Document Number: 96050774. PubMed ID: 7496917. Flow cytometric immunodetection of human immunodeficiency virus type 1 proviral DNA by heminested PCR and digoxigenin-labeled probes. Yang G; Garhwal S; Olson J C; Vyas G N. (Department of Laboratory Medicine, University of California, San Francisco 94143-0134, USA.) CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1994 Jan) 1 (1) 26-31. Journal code: 9421292. ISSN: 1071-412X. Pub. country: United States. Language: English.

AB PCR is the most sensitive and direct method for detecting blood-borne viruses, as well as an efficient means for producing vector-free probes. However, the application of PCR, especially in the laboratory diagnosis of human immunodeficiency virus (HIV) infection, is impeded by the current use of radiolabeled oligonucleotide probes. Therefore, we have developed a nonisotopic PCR immunoreactive bead (PCR-IRB) assay to detect HIV type 1 proviral DNA from peripheral blood mononuclear cells (PBMC). We used a biotinylated primer in a set of three oligonucleotides selected from the HIV long terminal repeat region for heminested PCR amplification. An internal probe was synthesized by PCR with incorporation of digoxigenin-labeled dUTP. After solution hybridization of the probe with PCR-amplified products (amplicons), the hybridized DNA was captured with streptavidin-coated magnetic beads. For the detection of hybrids, flow cytometric analyses were carried out by two procedures: (i) direct detection with fluorescein isothiocyanate (FITC)-labeled antidigoxigenin immunoglobulin G (IgG) antibody and (ii) indirect detection with antidigoxigenin sheep IgG antibody followed by FITC-labeled anti-sheep IgG antibody. Both procedures in the PCR-IRB assay detected two to three copies of HIV proviral DNA sequences, a sensitivity that is comparable with that of the conventional radioactive detection of amplicons following probe hybridization and electrophoresis. To compare the PCR-IRB assay with the conventional method, we tested 53 pedigreed PBMC specimens from blood donors and newborns; the results obtained were identical. This nonisotopic PCR-IRB assay can also be automated for potential application in laboratory diagnosis of HIV infection, blood bank screening, and therapeutic monitoring of viremia and perinatal transmission.

L48 ANSWER 28 OF 31 MEDLINE
91009729 Document Number: 91009729. PubMed ID: 2211956. Removal of HIV antigens and HIV-infected cells in vitro using

immunomagnetic beads. Ushijima H; Honma H; Tsuchie H; Kitamura T; Takahashi I. (Division of AIDS Virus, National Institute of Health, Tokyo, Japan.) JOURNAL OF VIROLOGICAL METHODS, (1990 Jul) 29 (1) 23-31. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB Human anti-HIV antibody-coated magnetic beads and magnetic particle concentrators were used to eliminate HIV antigens and the infected cells in vitro. Fifty micrograms/ml of the antibody coated on 30 mg/ml of tosyl-activated beads, and 10 min of reaction time between the antigens and the immunobeads were sufficient to eliminate the virus antigens from phosphate buffered saline, serum and peripheral blood. HIV-infected cells were eliminated in vitro, but not completely. This method will be useful in eliminating viral antigens and infected cells from clinical material such as blood and blood products.

L48 ANSWER 31 OF 31 MEDLINE

87302217 Document Number: 87302217. PubMed ID: 3113767. Application of novel chromium dioxide magnetic particles to immunoassay development. Birkmeyer R C; Diaco R; Hutson D K; Lau H P; Miller W K; Neelkantan N V; Pankratz T J; Tseng S Y; Vickery D K; Yang E K. CLINICAL CHEMISTRY, (1987 Sep) 33 (9) 1543-7. Journal code: 9421549. ISSN: 0009-9147. Pub. country: United States. Language: English.

AB We have used chromium dioxide magnetic particles as the solid support in developing a series of immunological tests. The high surface area (greater than 40 m2/g) available on the magnetic particles and their easy dispersion throughout a solution allow for rapid and complete capture of the target antigen. The magnetic responsiveness of the particles allows for rapid, high-efficiency washing to reduce nonspecific binding, which often limits the sensitivity of serological assays. These features form the basis of extremely rapid and flexible assays for several hormones and markers of cancer and infectious disease. Most of the assays involve monoclonal antibodies. Here we describe specific performance characteristics for thyroxin, follitropin, creatine kinase isoenzyme MB, and antibody to human immunodeficiency virus (HIV). All of the assays are performed in less than 90 min, many in 30 to 45 min. The technology is highly flexible and is suitable for a variety of formats, from manual to fully automated.

L50 ANSWER 6 OF 9 MEDLINE

95248283 Document Number: 95248283. PubMed ID: 7730799. CD4 expression on dendritic cells and their infection by human immunodeficiency virus. Patterson S; Gross J; English N; Stackpoole A; Bedford P; Knight S C. (Antigen Presentation Research Group, Clinical Research Centre, Harrow, Middlesex, UK.) JOURNAL OF GENERAL VIROLOGY, (1995 May) 76 (Pt 5) 1155-63. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Infection of dendritic cells (DC) by human immunodeficiency virus (HIV) has been disputed. Employing a fluorescence-activated cell sorter, DC, identified by the absence of membrane markers for T, B, natural killer (NK) and monocytic cells and by high levels of MHC class II DR antigen, were shown to express low levels of CD4. Immunomagnetic beads were used to separate blood low density cells, which are enriched for DC, into CD4-positive and -negative populations. Examination of these cells by electron microscopy showed an increase in the percentage of cells with DC morphology in the CD4-positive fraction and a reduction in the

CD4-negative fraction. Electron microscopy of semi-purified DC preparations infected in vitro for 5 days with HIV-1 revealed morphologically distinct veiled DC with mature virions on the cell surface and virus budding through the cell membrane. Further evidence for the growth of HIV in DC was provided by experiments in which DC were extensively depleted of contaminating lymphocytes and monocytes prior to infection. Estimation of provirus load by a nested PCR indicated that after 5 days an infection level of one provirus copy per five cells could be achieved. After 7 days the provirus copy number could exceed the cellular genome copy number, suggesting that some cells had more than one provirus. Infectious virus could not be demonstrated in these cultures after 24 h but was detected after 5 or 7 days. Infection of DC in the presence of antibodies against CD4 was inhibited and suggests infection occurs via a CD4-dependent pathway. These results confirm that DC are susceptible to HIV infection in vitro. The immunological consequences of DC infection in vivo may be significant in the pathogenesis of AIDS.

L50 ANSWER 5 OF 9 MEDLINE
1998043940 Document Number: 98043940. PubMed ID: 9384284. Immunomagnetic selection of purified monocyte and lymphocyte populations from peripheral blood mononuclear cells following cryopreservation. Sleasman J W; Leon B H; Aleixo L F; Rojas M; Goodenow M M. (Department of Pediatrics, University of Florida College of Medicine, Gainesville 32610, USA.) CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1997 Nov) 4 (6) 653-8. Journal code: 9421292. ISSN: 1071-412X. Pub. country: United States. Language: English.

AB Cryopreservation is a method commonly used to store human blood samples. We sought to determine if cryopreserved peripheral blood mononuclear cells (PBMC) could be separated effectively into distinct populations by using monoclonal antibodies and immunomagnetic microspheres. PBMC obtained from healthy blood donors and from human immunodeficiency virus-infected subjects were cryopreserved for as long as 18 months. Recovered cells were separated into CD14+ monocytes and CD4+ T-cell subsets by immunomagnetic selection. Flow cytometry analysis indicated >95% depletion of monocytes from PBMC following immunomagnetic selection with anti-CD14. A highly enriched population of CD4+ T cells was obtained from the CD14-depleted cell fraction by using an anti-CD4 monoclonal antibody and detachable immunomagnetic beads . The CD4+ T cells were subsequently separated into CD4+ CD45RO and CD4+ CD45RA fractions. Each fraction contained >90% enrichment for the respective subpopulation and <5% of the reciprocal subpopulation. No significant differences in cell surface expression of leukocyte markers, in efficiency of selection of PBMC subpopulations, or in mitogen-induced proliferation were detected in freshly isolated or cryopreserved cells. Efficient recovery of cryopreserved specimens means that targeted assays can be performed on selected, prospectively stored samples once clinical endpoints have been achieved.

L57 ANSWER 74 OF 115 MEDLINE
92249064 Document Number: 92249064. PubMed ID: 1374306. Detection of viral surface antigens on HIV-2ben infected human tumor cell lines by flow cytometry. Bohm D; Nick S; Voss G; Hunsmann G. (German Primate Centre, Gottingen.) CYTOMETRY, (1992) 13 (3) 259-66. Journal code: 8102328. ISSN: 0196-4763. Pub. country: United States. Language: English.

AB The human monocytic cell line U-937 clone 2 and two T-cell lines CEM and MOLT-4 clone 8 were infected with HIV-2ben, a recent isolate of HIV-2. Infection and subsequent antigen expression on the cell

surface was monitored by flow cytometry using a rabbit-anti-serum against tween-ether-treated HIV-2ben and a fluorescein-isothiocyanate-conjugated IgG against rabbit-IgG. The sensitivity of the three cell lines to infection with HIV-2ben correlated with the percentages of CD4-expressing cells but not with the levels of CD4-expression on the cell. The appearance of viral surface antigens preceded the formation of syncytia and correlated closely with the infecting virus dose. After 1-2 weeks in culture, 20-85% of the cells of each line expressed viral surface antigens. The variation depended on the cell type and cell culture conditions. The MOLT-4 clone 8 and the U-937 clone 2 cells died around 10 or 20 days, respectively, after HIV-2ben infection. Only HIV-2ben infected CEM cells grew permanently. Flow cytometry was an appropriate method to monitor the expression of viral proteins on the cell surface of HIV-infected cell lines. Flow cytometry proved to be more sensitive than determination of RT activity in supernatants of HIV-infected cells and more precise than light microscopy examinations.

L57 ANSWER 70 OF 115 MEDLINE
92348905 Document Number: 92348905. PubMed ID: 1640108. Evaluation of a flow cytometric model for monitoring HIV antigen expression in vitro. Heynen C A; Holzer T J. (Abbott Laboratories, Department of Experimental Biology Research, North Chicago, IL 60064.) JOURNAL OF IMMUNOLOGICAL METHODS, (1992 Jul 31) 152 (1) 25-33. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB Using flow cytometry, monoclonal antibodies to the HIV proteins p24, gp41 and p17 were evaluated for their ability to detect HIV antigens associated with HIV-infected T cells. Mixtures containing varying ratios of HIV-infected and uninfected cells were subjected to analysis with these monoclonal antibodies. In most cases, the monoclonal antibodies identified the correct ratio of HIV-infected cells to uninfected cells in the mixtures tested. An HIV anti-p24 monoclonal antibody was selected for further studies. Flow cytometric analysis was performed on various populations of cells including uninfected, acutely infected and chronically infected cells. Based on cell population fluorescence intensity three distinct regions were identified. In the first region were cells having low level fluorescence that were considered negative for HIV antigens, a profile detected in uninfected cells, and in the majority of cells in the first days following acute HIV infection. In the second region were those cells exhibiting strong fluorescence such as chronically infected cells or acutely infected cells several days after infection. A third region was identified containing cells that were intermediate in fluorescence intensity. Cells exhibiting intermediate intensity fluorescence appeared to have low concentrations of HIV p24 antigen associated with them either through viral adsorption and uptake or through low level virus expression. These intermediate region cells appeared in the early stages following acute infection, and also when chronically infected cells and uninfected cells were permeabilized together, suggesting a 'leaching' of HIV proteins from highly infected cells to uninfected cells. This leaching type of phenomenon could present problems in determining gating parameters for positive cells since uninfected cells that have associated HIV antigens exhibit higher fluorescence intensity than uninfected cells.

L68 ANSWER 7 OF 8 WPIDS (C) 2003 THOMSON DERWENT
AN 1998-261020 [23] WPIDS
CR 1998-129867 [12]; 2001-015870 [02]; 2001-015871 [02]; 2003-165403 [16]
DNN N1998-205788 DNC C1998-081010
TI Separating cell expressing particular antigen by reaction with specific binding pair - having one component bound to magnetic beads, specifically used for early diagnosis of infection by human immune deficiency virus.
DC B04 D16 S03
IN HALLOWITZ, R A; KING, C F; KING, C; YOUNG, S
PA (BIOT-N) BIO-TECH IMAGING INC; (HALL-I) HALLOWITZ R A; (KING-I) KING C F; (KING-I) KING C; (YOUN-I) YOUNG S; (AVRI-N) AVRIEL GROUP AMCAS DIV INC
CYC 80
PI WO 9816101 A1 19980423 (199823)* EN 55p
RW: AT BE CH DE DK EA ES FI FR GB GH GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 9748224 A 19980511 (199837)
US 5817458 A 19981006 (199847)
EP 933989 A1 19990811 (199936) EN
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
JP 2001503859 W 20010321 (200122) 48p
AU 2001028112 A 20010524 (200139)#
US 2001008760 A1 20010719 (200143)
AU 743937 B 20020207 (200224)
US 2002037498 A1 20020328 (200225)
ADT WO 9816101 A1 WO 1997-US18649 19971015; AU 9748224 A AU 1997-48224 19971015; US 5817458 A US 1996-732782 19961015; EP 933989 A1 EP 1997-910976 19971015, WO 1997-US18649 19971015; JP 2001503859 W WO 1997-US18649 19971015, JP 1998-518582 19971015; AU 2001028112 A Div ex AU 1997-48224 19971015, AU 2001-28112 20010319; US 2001008760 A1 WO 1997-US18649 19971015, US 1998-139663 19980825; AU 743937 B AU 1997-48224 19971015; US 2002037498 A1 CIP of US 1996-732782 19961015, CIP of US 1996-732784 19961015, Cont of WO 1997-US18649 19971015, Cont of US 1998-139633 19980825, US 1999-299625 19990427
FDT AU 9748224 A Based on WO 9816101; EP 933989 A1 Based on WO 9816101; JP 2001503859 W Based on WO 9816101; AU 743937 B Previous Publ. AU 9748224, Based on WO 9816101; US 2002037498 A1 CIP of US 5714390, CIP of US 5817458, Cont of US 6127490
PRAI US 1996-732784 19961015; US 1996-732782 19961015; AU 2001-28112 20010319; US 1998-139663 19980825; US 1998-139633 19980825; US 1999-299625 19990427

AB WO 9816101 A UPAB: 20030307
Cells that express a viral antigen (Ag) are separated by:
(i) infecting cells with a virus;
(ii) treating the cells with a first binding partner (BP1) specific for an Ag encoded by the virus and expressed on the cell surface;
(iii) adding a second binding partner (BP2), attached to a magnetic bead, to form a cell-Ag-BP1-BP2-bead complex, and
(iv) separating this complex by application of a magnetic field.
Also claimed are:
(1) a method for identifying agents (I) that interfere with viral infection by adding a test compound to the mixture formed in step (ii) and comparing the number of infected cells separated in the presence and absence of this compound;
(2) magnetic beads having a surface coated by a cell-specific receptor for human immune deficiency virus (HIV);

(3) similar methods for separating microorganisms or cancer cells that express a cell-surface Ag, and

(4) a cartridge antigen test system for collecting and testing blood in a single step.

USE - The method is particularly used to detect HIV and related viruses by identifying/separating cell expressing viral Ag, specifically for early diagnosis.

More generally it can be used to detect/separate, e.g. peripheral blood leucocytes, T cells, immortalised cell lines, macrophages or liposomes; to detect antibodies (against a viral antigen); to identify agents that modulate viral infection (e.g. ribozymes, antiviral agents or cytokines) or to isolate viruses from plasma.

ADVANTAGE - The use of magnetic particles eliminates the need for washing or removing unbound BPs. The new disposable device allows collection of blood and mixing with reagents in a single package and is adapted to be viewed on a fluorescent microscope, for quick and accurate testing of whole blood.

The device requires no transfers, additions or other manipulations, is easy to use and suitable for automation. Detecting HIV Ag, rather than antibodies, means that infection can be detected within 4 days of exposure, i.e. comparable to polymerase chain reaction methods but at a fraction of the cost.

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